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Chemically Defined Sialoside Scaffolds for Investigation of Multivalent Interactions with Sialic Acid Binding Proteins**

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Abstract: Four glycodendrons and a glycocluster were synthesized from carbohydrate building blocks to form paucivalent (di- to tetravalent) structures of controlled scaffold architectures. Enzymatic sialylation of the functionalized cluster and dendrons, terminated in lactose residues, generated a library of paucivalent synthetic sialosides displaying sialic acids with different dispositions. These newly constructed bioactive sialic acid-based structures were differentially recognized by sialoadhesin, a mammalian macrophage sialic acid binding protein. The binding of the sialosides to sialoadhesin was evaluated by an enzyme-linked immunosorbant assay to investigate the complementarity of scaffold structure and binding to sialoadhesin. Modulating the interaction between sialoadhesin and its sialic acid ligands has important implications in immunobiology.

Supporting Information
(33 pages)

^{1}H NMR for compounds **3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 16**
^{13}C NMR for compounds **3, 4, 5, 6, 11, 12, 13, 16**
^{13}C DEPT NMR for compounds **3, 11, 12, 13, 16**

General Considerations. Chemicals were purchased from commercial suppliers and used as received. The ST3-fusion protein[25a] and CMP-Neu5Ac[25b] were prepared following literature procedures. All reactions were performed glass round-bottomed flasks with the following exceptions: photoadditions were done in borosilicate glass vials and enzymatic reactions were carried out in snap-cap microcentrifuge tubes. Thin-layer chromatography (TLC) was carried out on aluminum sheets, coated with silica-gel 60 F and glass plates, coated with Kieselgel 60 F_{254}. The plates were inspected by UV light and developed by treatment with an orcinol reagent containing a mixture of 5% H_2SO_4 in EtOH or an anisaldehyde reagent, followed by heating. Preparative reverse-phase chromatography was conducted on fully end-capped C-18, or C-8 silica gel 100 (230–400 mesh), and gel permeation chromatography (GPC) was performed using a 25×900 mm column of Sephadex LH20 resin, eluting with MeOH, or a 25×1400 mm column of Sephadex G-25 Fine, eluting with 5% *n*BuOH in H_2O. Analytical reverse-phase HPLC was conducted using a Hypersil 5 mm BDS C-18 silica column (4.6×250 mm) under isocratic elution conditions (MeOH/H_2O/TFA, 65:35:0.0001) with UV detection, using a Dynamax PDA-2 diode array detector. ^1H and ^{13}C NMR spectra were recorded on 400 or 500 MHz spectrometers using the residual solvent or TMS as the internal standard. The chemical shifts are expressed on the d scale in parts per million (ppm). The following abbreviations are used to explain the observed multiplicities: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; br, broad.

Pentaerythritol-*tetrakis*-[(tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-thioglucopyranosyl)-3-propyl] tetraether (3). Peracetylated β-thiolactose[34] (391 mg, 600 μmol) and tetra-*O*-allyl-pentaerythritol[35] (15 mg, 50 μmol) were dissolved in freshly distilled MeOH (10 mL). Argon was bubbled through the solution for 30 min to thoroughly degas the

solvent. The vial was then filled with argon, sealed and then irradiated with a Hg lamp and stirred for 5 h. The reaction mixture was concentrated to a smaller volume and the resulting solution was purified using gel filtration chromatography (LH-20, MeOH) to give **3** (65 mg, 45%) after freeze-drying. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.73–1.82 (m, 8H, 4×SCH_2CH_2), 1.95, 2.04, 2.05, 2.10, 2.14 (5s, 84H, 21×COMe), 2.66–2.70 (m, 8H, 4×SCH_2), 3.28, 3.32 (2t, J = 5.8 Hz, 16H, 8×OCH_2), 3.63 (m, 4H, H-5b), 3.77 (t, J = 9.4 Hz, 4H, H-6b), 3.88 (m, 4H, H-5a), 4.05–4.13 (m, 16H, H-6a, H-6a', H-4a, H-6b'), 4.45–4.50 (m, 12H, H-3a, H-1b, H-1a), 4.88 (m, 4H, H-2a), 5.07–5.11 (m, 4H, H-4b), 5.18–5.21 (t, J = 9.2 Hz, 4H, H-2b), 5.33–5.34 (d, J = 2.6 Hz, 4H, H-3b); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.4, 20.5, 20.6 (3C), 20.7, 20.8, 27.4, 29.9, 60.6, 62.2, 66.5, 69.0, 69.3, 70.3, 70.5, 70.9, 73.7, 76.1, 76.5, 76.7, 83.7, 101.0, 169.0, 169.5, 169.6, 169.9, 170.0, 170.1 (2C), 170.2. MS (MALDI-TOF) m/z 2927.76 $[M + Na]^+$.

Pentaerythritol-*tetrakis*-[(β-D-galactopyranosyl-(1→4)-β-D-thioglucopyranosyl)-3-propyl] tetraether (4). NaOMe (1 mL, 0.5 M in MeOH, 0.5 mmol) was added to a methanolic (5 mL of MeOH) solution of the peracetylated derivative **3** (65 mg, 22 μmol), and the mixture was left to stir at room temperature overnight. The solution was then neutralized with Amberlite IR-120 (H^+ form) ion exchange resin, filtered and concentrated to yield **4** (35 mg, 90%). ^{1}H NMR (D_2O, 500 MHz): δ 1.94 (bs, 8H, 4×SCH_2CH_2), 2.70–2.85 (m, 8H, 4×SCH_2), 3.39–3.99 (band of m, 64H, 8×OCH_2, H-2a, H-3a, H-4a, H-5a, H-6a, H-6a', H-2b, H-3b, H-4b, H-5b, H-6b, H-6b'), 4.46–4.47 (d, 4H, $J_{1b,2b}$ = 7.6 Hz, H-1b), 4.56–4.58 (d, 4H, $J_{1a,2a}$ = 9.9 Hz, H-1a); ^{13}C NMR (D_2O, 125 MHz): δ 26.7, 29.1, 60.2, 61.0, 68.5, 69.1, 69.9, 70.9, 72.0, 72.5, 75.3, 75.7, 78.1, 78.6, 85.3, 102.8, 170.1. MS (MALDI-TOF) m/z 1751.52 $[M + Na]^+$.

General Procedure for the Synthesis of Sialosides 5, 7, 9, 14, and 17. The lactoside (6.9 μmol) and CMP-Neu5Ac (88 mg, 83 μmol) were dissolved in H_2O (1.2 mL) containing ST3-

Fusion[25] (2.0 mL, 11 U), $MnCl_2$ (0.4 mL, 200 mM), and cacodylate buffer (0.4 mL, 500 mM, pH = 6.6). The pH of the reaction was maintained between 6 and 7 and the progress of the reaction was monitored by TLC (EtOAc/MeOH/AcOH/H_2O, 4:3:3:2). After 1 day, the reaction mixture was centrifuged to remove some of the excess of the fusion protein and the supernatant was further purified by gel filtration chromatography (Sephadex G25, 95% H_2O, 5% *n*BuOH) to afford the target sialoside.

Pentaerythritol-*tetrakis*-[(5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2 nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-thioglucopyranosyl)-3-propyl] tetraether (5). ^{1}H NMR (D_2O, 500 MHz): δ 1.66 (t, 4H, $J_{3eqc,3axc}$ = 12.1 Hz, H-3_{ax}c), 1.80 (t, 8H, *J* = 6.5 Hz, 4×SCH_2), 2.60–2.70 (m, 24 H, 4×SCH$_2$CH_2, 4×NHCOCH_3, and 4×H-3_{eq}c), 3.29–3.70 (band of m, 80 H, 8×OCH_2, H-2a, H-3a, H-4a, H-5a, H-6a, H-6a', H-2b, H-5b, H-6b, H-6b', H-5c, H-6c, H-7c, H-8c, H-9c, H-9c'), 3.81 (d, 4H, $J_{4c,3c}$ = 2.7 Hz, H-4c), 3.92 (d, 4H, *J* = 3.0 Hz, H-4b), 3.96 (dd, 4H, *J* = 10.0 Hz, H-3b), 4.32 (d, 4H, J = 8.1 Hz, H-1a), 4.39 (d, 4H, *J* = 7.7 Hz, H-1b); ^{13}C NMR (D_2O, 125 MHz): δ 21.9, 26.6, 29.0, 39.4, 51.5, 60.1, 60.9, 62.4, 67.3, 67.9, 68.2, 68.9, 69.2, 69.4, 69.7, 71.6, 71.9, 72.7, 75.0, 75.3, 75.6, 77.9, 78.5, 85.2, 99.7, 102.4, 173.8, 174.8. MALDI-FTMS *m/z* Calcd for $C_{109}H_{184}N_4O_{76}S_4Na$ $[M + Na]^+$ 2917.0215. Found 2917.0215.

Bis-[(5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-thioglucopyranosyl-3-propyl]-6,6'-β-maltosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (7). Selected ^{1}H NMR (D_2O, 500 MHz) data: δ 1.27, 1.35, 1.45, 1.46 (4s, 12H, 2×CMe_2), 1.66 (t, 2H, *J* = 12.0 Hz, H-3_{ax}f, H-3_{ax}i), 1.82 (m, 4H, 2×SCH_2), 1.89 (s, 6H, 2×NHCOCH_3), 2.62 (m, 6H, H-3_{eq}f, H-3_{eq}i, 2×SCH$_2$CH_2), 3.23 (m, 3H, H-2b, H-2d, H-2g), 3.33 (m, 1H, 4b), 3.90 (m, 2H, H-4e, H-4h), 3.92 (m, 2H, H-3e, H-3a), 4.40 (m,

5H, H-1b, H-1d, H-1e, H-1g, H-1h), 5.19 (d, 1H, $J_{1c,2c}$ = 3.7, H-1c), 5.54 (d, 1H, $J_{1a,2a}$ = 4.9, H-1a); ^{13}C NMR (D_2O, 125 MHz): δ 21.9, 23.1, 24.7, 24.8, 39.5, 51.5, 60.9, 62.4, 67.3, 67.9, 68.2, 69.2, 69.3, 69.4, 69.7, 69.8, 71.6, 71.9, 72.7, 75.0, 75.3, 75.6, 78.5, 85.3, 95.6, 99.6, 102.5, 109.8, 109.9, 173.7, 174.8. MALDI-FTMS *m/z* Calcd for $C_{76}H_{126}N_2NaO_{52}S_2$ [M + Na]$^+$ 1986.1662. Found 1986.1661.

Bis-[(5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-thioglucopyranosyl-3-propyl]-6,6'-β-maltosyl-(1→6)-D-galactose (9). Selected ^{1}H NMR (D_2O, 500 MHz) data: δ 1.66 (t, 2H, *J* = 12.0 Hz, H-3_{ax}f, H-3_{ax}i), 1.82 (m, 4H, 2×SCH_2CH_2), 1.89 (s, 6H, 2×$NHCOCH_3$), 2.62 (m, 6H, H-3_{eq}f, H-3_{eq}i, 2×SCH_2), 3.23 (m, 3H, H-2b, H-2d, H-2g), 3.63 (m, 2H, H-4f, H-4i), 3.90 (m, 2H, H-4e, H-4h), 3.92 (m, 2H, H-3e, H-3f), 4.40 (m, 5H, H-1b, H-1d, H-1e, H-1g, H-1h), 5.19 (d, 1H, $J_{1f,2f}$ = 3.7, H-1f); ^{13}C NMR (D_2O, 125 MHz): δ 13.9, 21.9, 26.6, 29.1, 29.5, 31.5, 31.6, 31.9, 37.5, 39.5, 51.5, 56.7, 60.1, 60.9, 62.4, 67.3, 67.9, 68.2, 69.1, 69.2, 69.3, 69.4, 69.6, 69.7, 71.6, 71.09, 72.7, 73.6, 75.0, 75.3, 75.6, 77.9, 78.5, 85.2, 92.2, 96.3, 99.8, 102.3, 102.4, 102.7, 113.7, 126.6, 173.7, 174.8. MALDI-FTMS *m/z* Calcd for $C_{70}H_{118}N_2NaO_{52}S_2$ [M + Na]$^+$ 1906.9993. Found 1907.0001.

β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (11). A solution of **10** (21.8 g, 24.8 mmol) and NaOMe (1.5 mL, 0.5 M in MeOH, 0.75 mmol) in MeOH (150 mL) was stirred for 6 h at room temperature. The solution was neutralized with Amberlite IR-120 (H^+ form) ion exchange resin, filtered and concentrated to afford **11** (12.02 g, 83%) as a foam. ^{1}H NMR (CD_3OD, 500 MHz): δ 1.29–1.49 (4s, 12H, 2×CMe_2), 3.24 (dd, $J_{2b,1b}$ = 7.8 Hz, $J_{2b,3b}$ = 8.35 Hz, 1H, H-2b), 3.40 (ddd, $J_{5b,6b'}$ = 2.3 Hz, $J_{5b,6b}$ = 4.4 Hz, $J_{5b,4b}$ = 9.8 Hz, 1H, H-5b), 3.47 (t, 1H, H-4b), 3.48–3.60 (m, 4H, H-2c, H-3c, H-5c, H-3b), 3.62 (m, 1H, H-6a), 3.67 (dd, $J_{6c,5c}$ = 4.6 Hz, $J_{6c,6c'}$ = 11.4 Hz, 1H, H-6c), 3.74 (dd, $J_{6c',5c}$ = 7.5

Hz, 1H, H-6c'), 3.78–3.81 (m, 1H, H-4c), 3.80 (dd, $J_{6b,6b'}$ = 12.2 Hz, 1H, H-6b), 3.88 (dd, 1H, H-6b'), 3.98–4.03 (m, 2H, H-5a, H-6a'), 4.28 (dd, $J_{4a,5a}$ = 1.3 Hz, $J_{4a,3a}$ = 7.9 Hz, 1H, H-4a), 4.30 (d, $J_{1b,2b}$ = 7.8 Hz, 1H, H-1b), 4.31 (d, $J_{1c,2c}$ = 7.4 Hz, 1H, H-1c), 4.33 (dd, $J_{2a,3a}$ = 2.3 Hz, $J_{2a,1a}$ = 5.0 Hz, 1H, H-2a), 4.60 (dd, 1H, H-3a), 5.48 (d, 1H, H-1a); ^{13}C NMR (CD_3OD, 125 MHz): δ 24.5, 25.1, 26.3 (2C), 61.9, 62.4, 68.8, 69.8, 70.2, 71.8, 71.85, 72.35, 72.4, 74.5, 74.7, 76.0, 76.4, 77.0, 80.6, 97.6, 104.5, 105.0, 110.0, 110.4. FAB-MS *m/z* 607.46 $[M + Na]^+$, 585.49 $[M + H]^+$, 569.42 $[M - CH_3]^+$. HRFAB-MS *m/z*: Calcd for $C_{24}H_{41}O_{16}$ $[M + H]^+$ 585.2395. Found 585.2386.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→6)-1,2,3,4-tetra-*O*-acetyl-D-galactopyranose (12). Diacetonide **11** (11.16 g, 19.11 mmol) was dissolved in TFA/H_2O (100 mL, 9:1) and stirred at room temperature for 1 h. The solution was concentrated then co-evaporated with MeOH (200 mL). The resulting foam was dissolved in H_2O (50 mL) and freeze dried. The crude product was then dissolved in C_5H_5N (50 mL) before adding DMAP (50 mg) and acetic anhydride (25 mL). Reaction was stirred at room temperature for 16 h before being poured into 1 M HCl (500 mL). The aqueous layer was extracted with CH_2Cl_2 (3×500 mL). The organic layer was then washed with satd. aq. $NaHCO_3$ (2×500 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. Column chromatography (SiO_2, CH_2Cl_2 to CH_2Cl_2/EtOAc, 1:1) gave **12** (15.15 g, 50%) as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.92–2.16 (m, 33H, 11×COMe), 3.25–3.50 (m, 2H, H-6a, H-5b or H-5c), 3.67–3.72 (m, 1H, H-6a'), 3.73–3.79 (m, 1H, H-5b or H-5c), 3.85 (m, 1H, H-4b), 3.97 (m, 0.6H, H-5aβ), 4.00–4.14 (m, 3H, H-6b, H-6c, H-6c'), 4.22 (m, 0.4H, H-5aα), 4.41–4.49 (m, 3H, H-1b, H-1c, H-6b'), 4.79–4.84 (m, 1H, H-2b), 4.90–4.96 (m, 1H, H-3c), 5.02–5.18 (m, 3H, H-4a, H-2c, H-3b), 5.25–5.30 (m, 1H, H-2a), 5.32 (m, 1H, H-4c), 5.38 (m, 0.6H, H-3aβ), 5.46 (m, 0.4H, H-3aα), 5.66 (d, $J_{1a,2a}$ = 2.41 Hz, 0.6H, H-1aβ), 6.32 (d, $J_{1a,2a}$ = 8.33

Hz, 0.4H, H-1aα); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.6–21.0 (11C), 60.9, 61.9, 66.2, 66.7, 67.0 + 67.6 (1C), 67.1 + 67.8 (1C), 69.2, 69.9 + 72.83 (1C), 70.8, 71.03 + 72.79 (1C), 71.08, 71.37 + 71.42 (1C), 72.71, 72.74, 76.1, 89.7 + 92.2 (1C), 101.0, 101.1, 169.1–170.5 (11C). FAB-MS *m/z* 989.12 [M + Na]$^+$. HRFAB-MS *m/z* Calcd for $C_{40}H_{54}NaO_{27}$ [M + Na]$^+$ 989.2750. Found 989.2748.

β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-D-galactopyranose (13). A solution of 12 (11.0 g, 11.39 mmol) and NaOMe (5 mL, 0.5 M in MeOH) in MeOH (150 mL) was stirred for 3 h at room temperature. Formation of a pale yellow solid was observed. The reaction mixture was then filtered and the filtrate evaporated, affording more solid which was filtered and washed with MeOH. After three recovery operations from the filtrate, **13** (4.235 g, 74%) was obtained as a pale yellow powder. ^{1}H NMR (D_2O, 500 MHz): δ 3.20–4.15 (m, 12H, H-2, H-3, H-4, H-5, H-6, H-6'), 4.33–4.48 (m, 2.67H, H-1aβ, H-1b, H-1c), 5.15 (d, $J_{1a,2a}$ = 3.8 Hz, 0.33H, H-1aα); ^{13}C NMR (D_2O, 125 MHz): δ 59.8, 60.8 (2C), 68.0–78.2 (12C), 69.3, 92.3 + 96.5 + 102.7 (1C), 102.2. ES-MS *m/z* 1031.3 [2M + Na]$^+$, 527.0 [M + Na]$^+$, 505.1 [M + H]$^+$. HRMALDI-MS *m/z* Calcd for $C_{18}H_{32}NaO_{16}$ [M + Na]$^+$ 527.1588. Found 527.1591.

(5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-D-galactopyranose (14). ^{1}H NMR (D_2O, 500 MHz): δ 1.65 (t, $J_{3axd,\ 3eqd}$ = 12.0 Hz, 1H, H-3$_{ax}$d), 1.88 (s, 3H, NHCOCH_3), 2.60 (dd, $J_{3eqd,4d}$ = 4.1 Hz, $J_{3eqd,\ 3exd}$ = 12.0 Hz, 1H, H-3$_{eq}$d), 3.20 (br t, 1H, H-2b), 3.45–3.93 (m, 22H, H-2a, H-3a, H-4a, H-5a, H-6a, H-6a', H-3b, H-4b, H-5b, H-6b, H-6b', H-2c, H-5c, H-6c, H-6c', H-4d, H-5d, H-6d, H-7d, H-8d, H-9d, H-9d'), 3.95 (d, $J_{4c,3c}$ = 3.3 Hz, 1H, H-4c), 4.15 (br s, 1H, H-3c), 4.38 (br s, 1H, H-1b), 4.44 (br s, 1H, H-1c), 5.15 (br s, 1H, H-1a); ^{13}C NMR (D_2O, 125 MHz): δ 21.9, 22.0, 39.2, 39.4, 51.5, 52.1, 60.9, 62.4, 67.9, 68.6, 69.1, 69.4, 71.6, 72.4, 72.6, 72.7, 73.6,

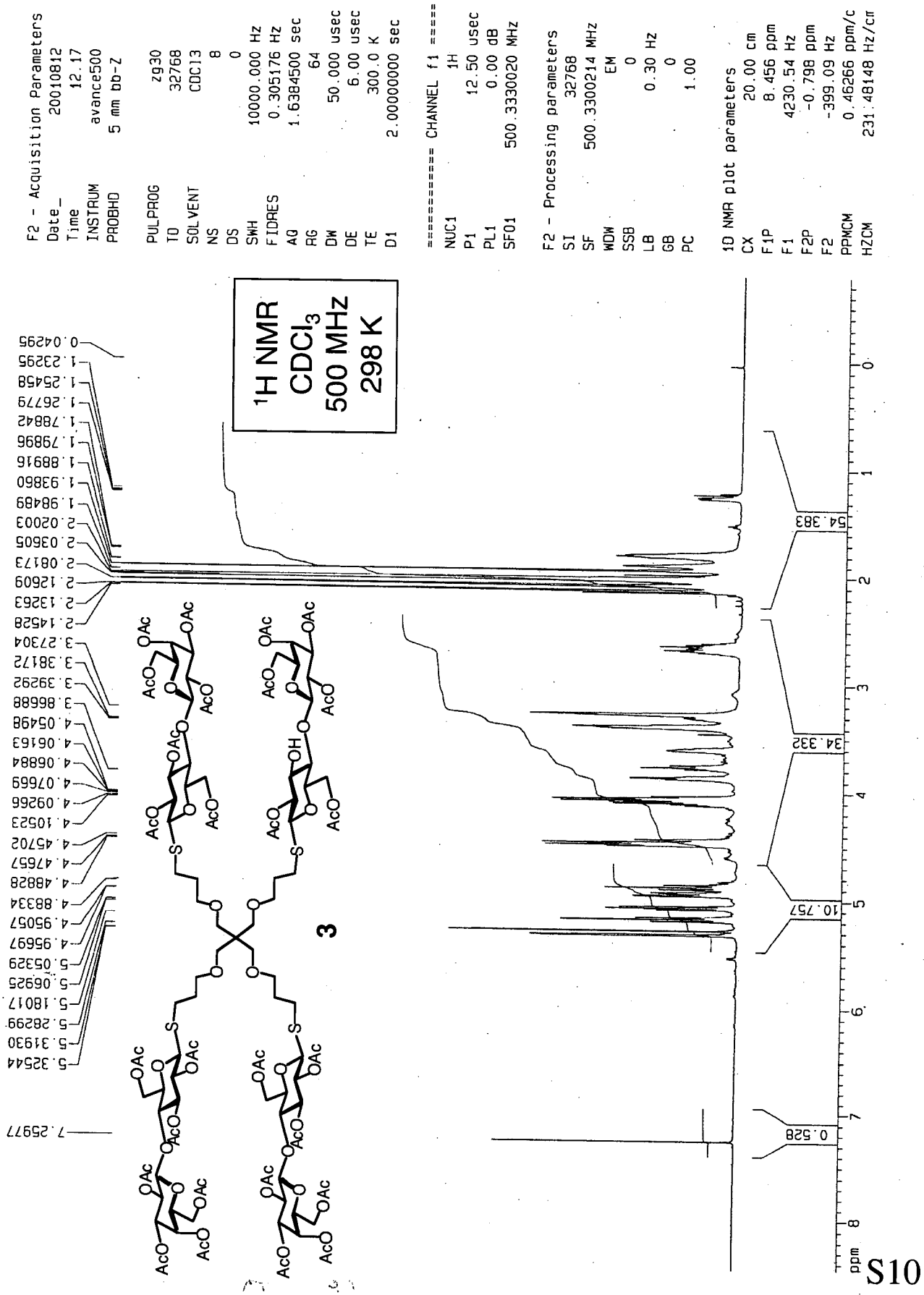
74.0, 74.6, 75.0, 75.3, 78.0, 92.1 + 102.2 (1C), 96.2, 99.6, 102.3, 102.5, 173.7, 174.8. MALDI-FTMS *m/z* Calcd for $C_{29}H_{49}NaO_{24}$ $[M + Na]^+$ 818.2537. Found 818.2537. HRESI *m/z* Calcd for $C_{29}H_{48}O_{24}$ $[M - H]^-$ 794.2566. Found 794.2590.

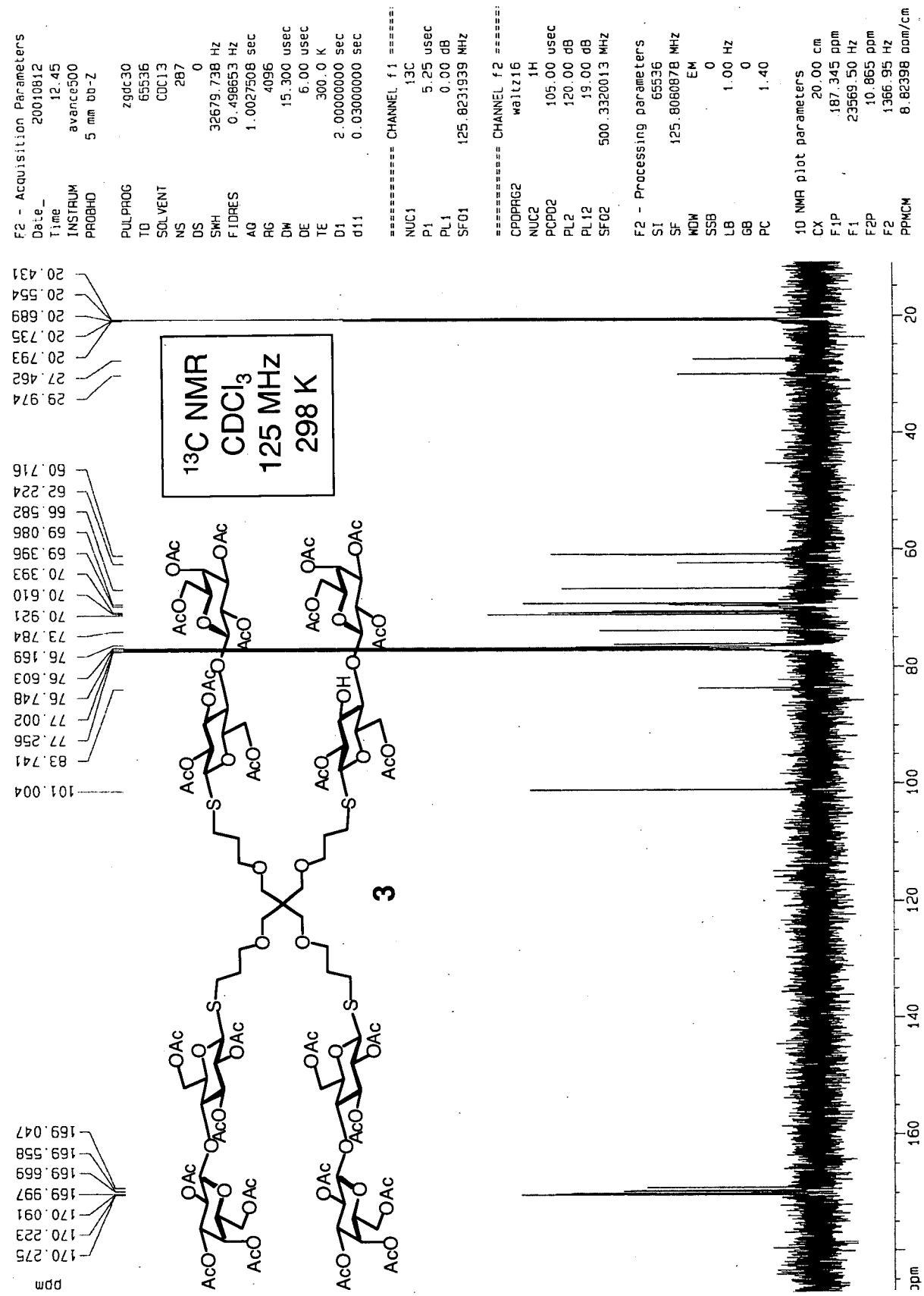
Bis-[β-lactosyl-(1→6)-D-galactit-1-yl]-6,6'-dideoxy-6,6'-dimethylamino-β-cellobiosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (16). Acetic acid (28 μL, 570 μmol) was added to a solution of the reducing sugar **13** (430 mg, 850 μmol), the *bis*-methylamino monomer **15** (200 mg, 320 μmol) and sodium cyanoborohydride (131 mg, 2.1 mmol) in MeOH (20 mL). The reaction mixture was stirred and heated under reflux for 6 h. The mixture was allowed to cool to room temperature, concentrated, and redissolved in H_2O (3 mL) and purified by preparative reverse-phase chromatography (15 g C-18 reverse-phase, MeOH/H_2O, 0:100 to 100:0) to afford pure **16** (254 mg, 50%). Selected ^{1}H NMR (CD_3OD, 500 MHz) data: δ 1.41, 1.42, 1.49, 1.62 (4s, 12H, 2×CMe_2), 2.78 (br s, 3H, NMe), 2.92 (br s, 3H, NMe), 3.08 (m, 2H, H-4e, H-4i), 4.01 (m, 1H, H-2a), 4.14–4.31 (m, 6H, H-1b, H-1c, H-1e, H-1f, H-1h, H-1i), 4.60 (dd, $J_{3a,2a}$ = 2.2 Hz, $J_{3a,4a}$ = 7.9 Hz, 1H, H-3a), 5.51 (d, $J_{1a,2a}$ = 4.9 Hz, 1H, H-1a); ^{13}C NMR (D_2O, 125 MHz): δ 23.0, 23.8, 24.6, 24.8, 43.5 (2C), 58.4 (6C), 59.8, 60.8 (2C), 67.4, 68.3, 69.3, 69.7, 70.3, 70.4, 70.7, 70.9, 71.7, 72.3, 72.6, 72.9, 74.1, 75.2, 78.2 (3C), 95.6, 102.0, 102.4, 102.8, 109.8, 109.9, 115.0. ESMS *m/z* 1588.8 $[M + H]^+$, 794.7 $[M + 2H]^{+2}$.

Bis-[(5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-D-galactit-1-yl]-6,6'-dideoxy-6,6'-dimethylamino-β-cellobiosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (17). Selected ^{1}H NMR (D_2O, 500 MHz) data: δ 1.25, 1.33, 1.44, 1.46 (4s, 12 H, 2×CMe_2), 1.66 (d, *J* = 12.0 Hz, 2H, H-3_{ax}g, H-3_{ax}k), 2.60 (d, *J* = 12.0 Hz, 2H, H-3_{eq}g, H-3_{eq}k), 2.52–2.82 (m, 8H, H-3_{eq}g, H-3_{eq}k, 2×NMe), 3.81 (d, *J* = 2.7 Hz, 2H, H-4g, H-4k), 3.92 (d, *J* = 3.0 Hz, 2H, H-4f, H-4j),

3.96 (d, J = 10.0 Hz, 2H, H-3f, H-3j), 4.41 (m, 6H, H-1b, H-1c, H-1e, H-1f, H-1i, H-1j), 5.53 (d, $J_{1a,2a}$ = 4.9, 1H, H-1a); ^{13}C NMR (D_2O, 125 MHz): δ 21.9 (2C), 31.6, 39.2, 39.4, 51.5, 52.1, 60.9, 62.4, 63.1, 67.1, 67.3, 67.9, 68.1, 68.4, 69.2, 69.4, 69.5, 70.0, 70.1, 71.6, 72.7, 74.6, 75.0, 75.3, 95.7, 96.2, 99.6, 102.0, 102.4, 102.5, 109.8, 109.9, 113.7, 173.7, 174.8. MALDI-FTMS *m/z* Calcd for $C_{84}H_{144}N_4O_{60}$ $[M + H]^+$ 2171.3433. Found 2171.3433.

General Method for ELISA. Protein A (1 μg/well) was immobilized on the wells of microtiter plates through incubation at 4 °C in hydrogen carbonate buffer (100 μL, 50 mM, pH 9.0). ELISA-buffer (200 μL, 20 mM HEPES and 0.5% Bovine Serum Albumin, 125 mM NaCl, pH 7.50) was added and remained for 1 h at room temperature to block the wells. After washing the wells with ELISA-buffer (5×200 μL), mSiglec-1-Fc (0.25 μg/well) that had been treated first with Neuraminidase (*Vibrio cholerae*, 50 mU/mL, 4 mM $CaCl_2$, ELISA-buffer) and then diluted with more ELISA-buffer was added to the wells. After washing the wells with ELISA-buffer (5×200 μL), the sialosides (50 μL, 5 mM–4 nM) were added to the wells. A solution of sialoside, SA-AP conjugate (50 mL, 1 μg/well) and biotinylated sialyllactose probe (0.5 μg/well) was added. After 20 min of incubation at room temperature, the wells were washed with ELISA-buffer (5×200 μL) and developed with *p*-nitrophenyl phosphate (50 μL/well). A plate reader was used to read the absorbances of the wells at 405 nm. Wells without mSiglec-1-Fc were used as negative controls and all assays were done in duplicate. To analyze the data, the inhibition curves used to generate IC_{50} values were fitted with a non-linear regression.

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DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
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PL1 0.00 dB
SFO1 125.8231939 MHz
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NUC2 1H
PCPD2 105.00 usec
PL2 120.00 dB
PL12 19.00 dB
SFO2 500.3320013 MHz
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SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
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F1 23569.50 Hz
F2P 10.865 ppm
F2 1366.95 Hz
PPMCM 8.82398 ppm/cm
13C NMR
CDCl3
125 MHz
298 K
20.431
20.554
20.689
20.735
20.793
27.462
29.974
60.716
62.224
66.582
69.086
69.396
70.393
70.610
70.921
73.784
76.169
76.603
76.748
77.002
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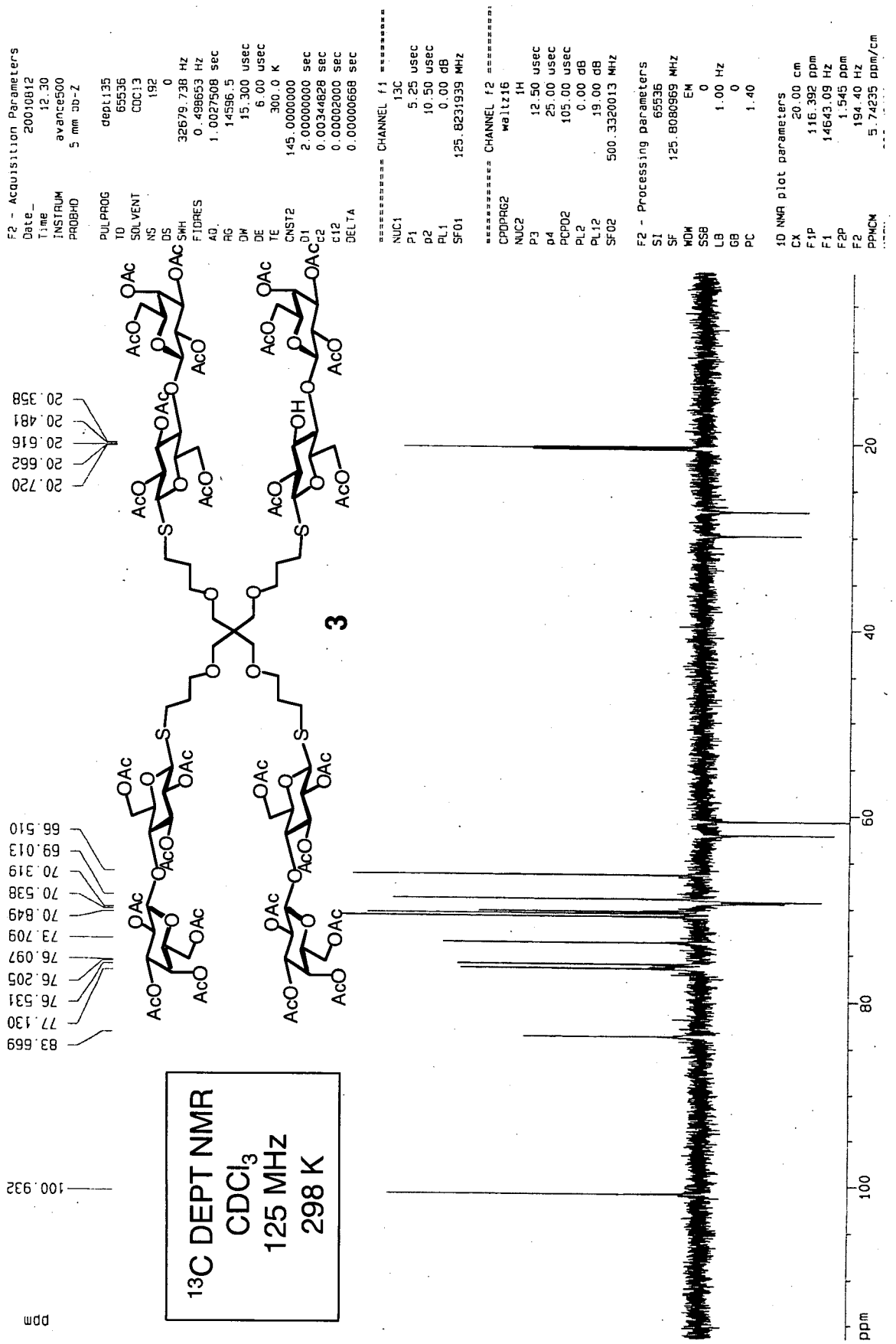
101.004
169.047
169.558
169.669
169.997
170.091
170.223
170.275
3
ppm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AQ 1.0027508 sec
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TE 300.0 K
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p2 10.50 usec
PL1 0.00 dB
SF01 125.8231939 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
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p4 25.00 usec
PCPD2 105.00 usec
PL2 0.00 dB
PL12 19.00 dB
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SSB 0
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F1 14643.09 Hz
F2P 1.545 ppm
F2 194.40 Hz
PPMCM 5.74235 ppm/cm
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20.481
20.616
20.662
20.720
66.510
69.013
70.319
70.538
70.849
73.709
76.097
76.205
76.531
77.130
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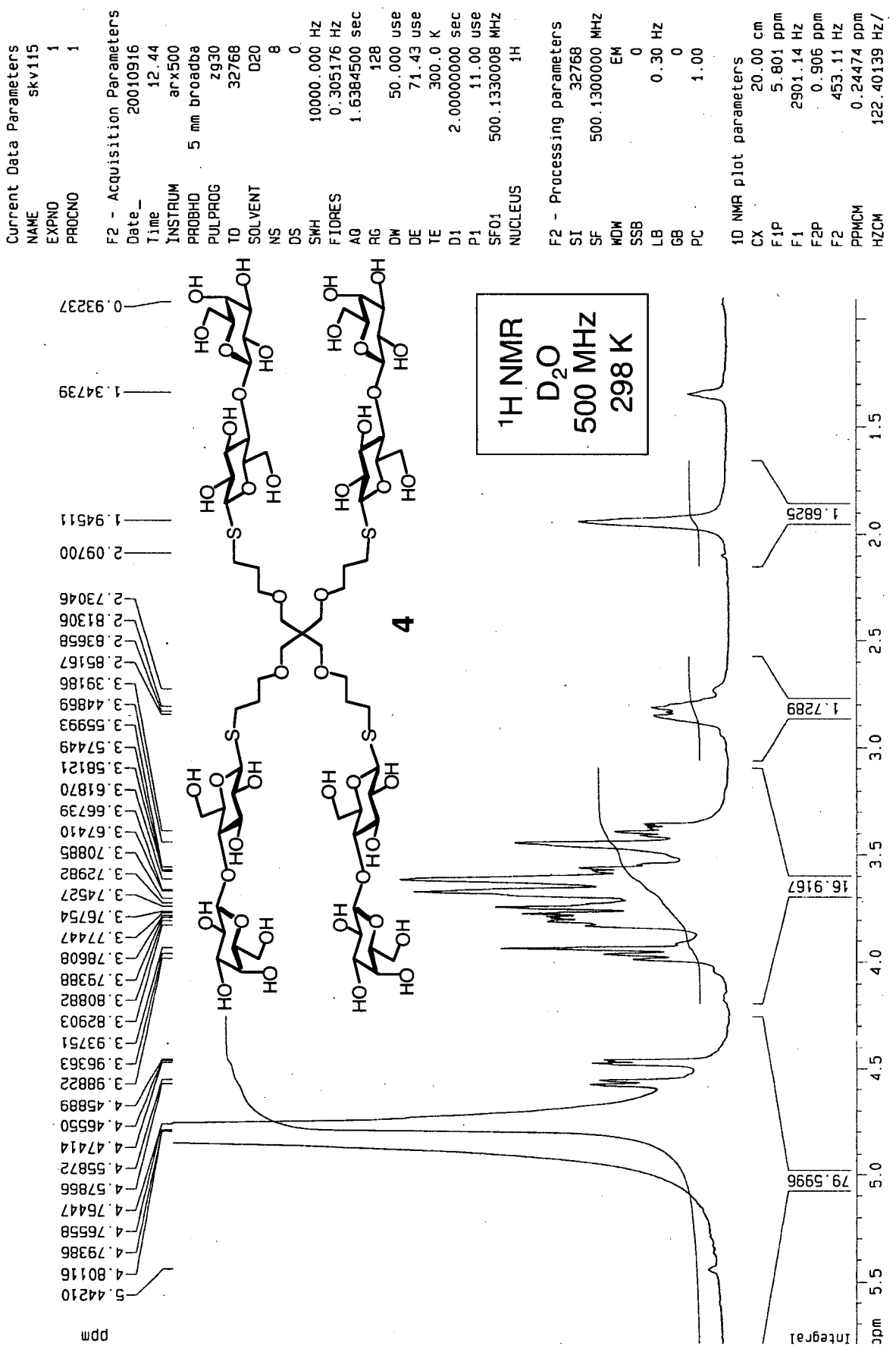
3
^{13}C DEPT NMR
$CDCl_3$
125 MHz
298 K
ppm
20
40
60
80
100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FIDRES 0.305176 Hz
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RG 128
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DE 71.43 use
TE 300.0 K
D1 2.00000000 sec
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NUCLEUS 1H
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LB 0.30 Hz
GB 0
PC 1.00
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HZCM 122.40139 Hz/
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1.94511
2.09700
2.73046
2.81306
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3.44869
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3.61870
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3.77447
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3.98822
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4.46550
4.47414
4.55872
4.57866
4.76447
4.76558
4.79386
4.80116
5.44210
ppm
4
1H NMR
D2O
500 MHz
298 K
1.6825
1.7289
16.9167
79.5996
Integral
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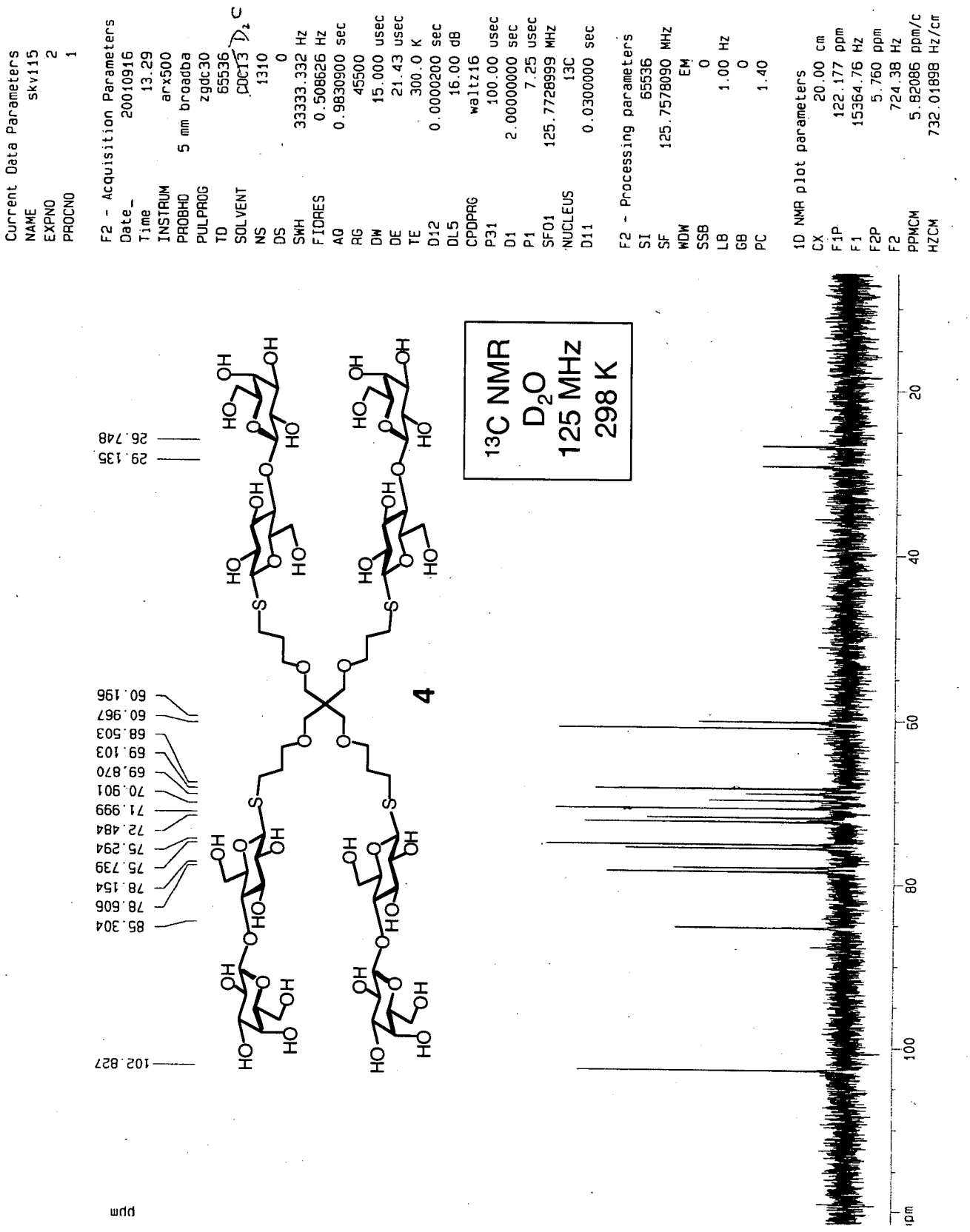
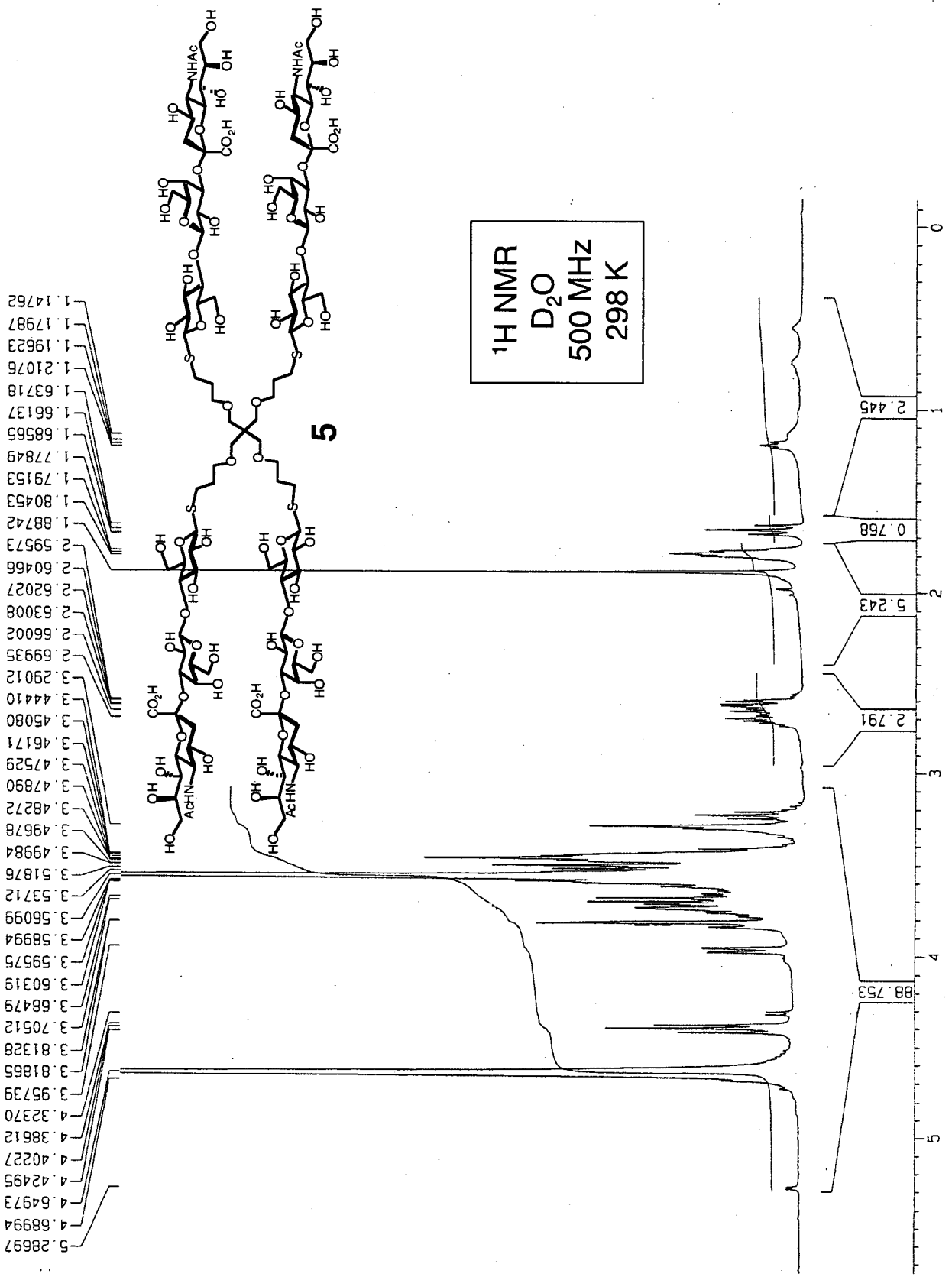
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm

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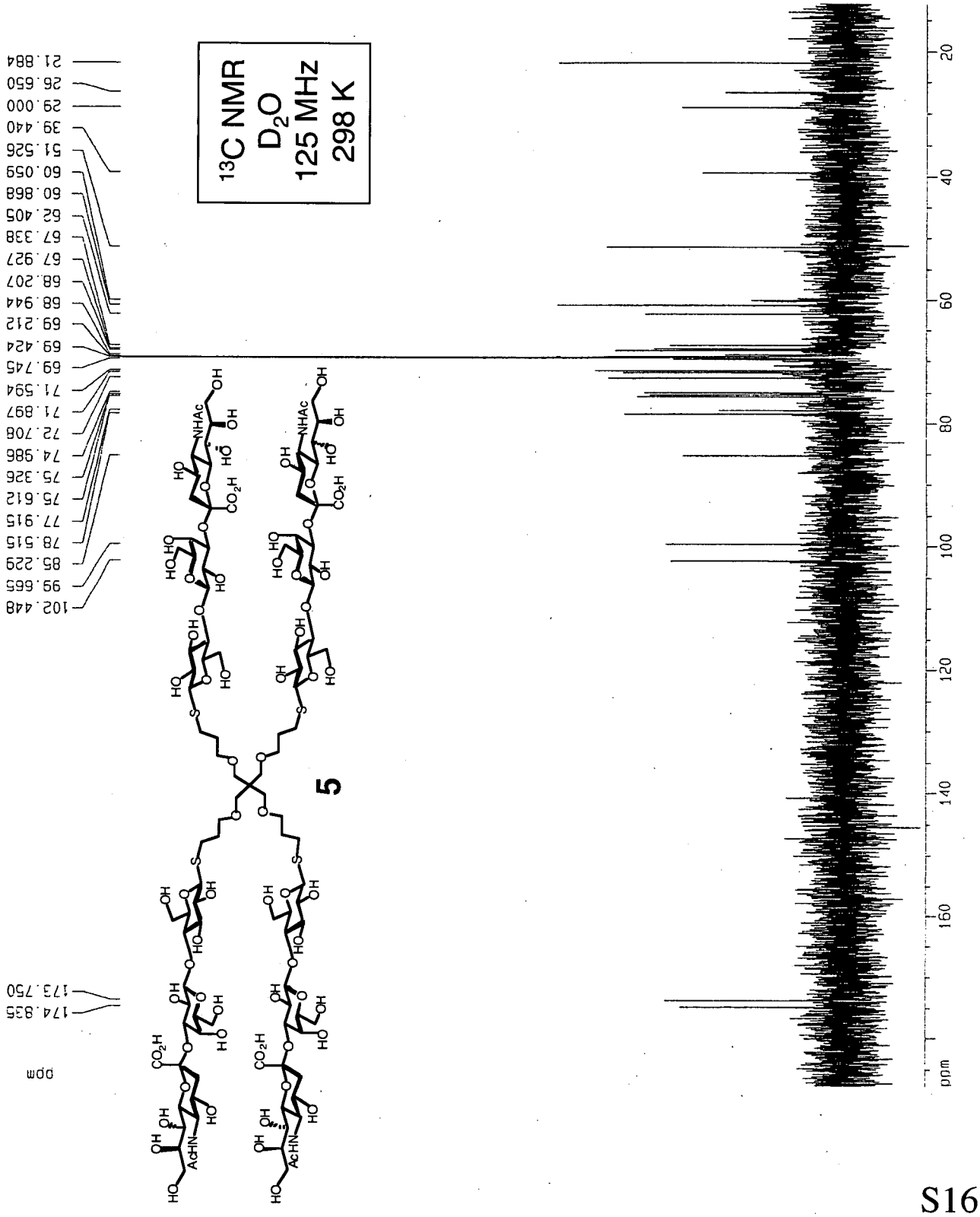
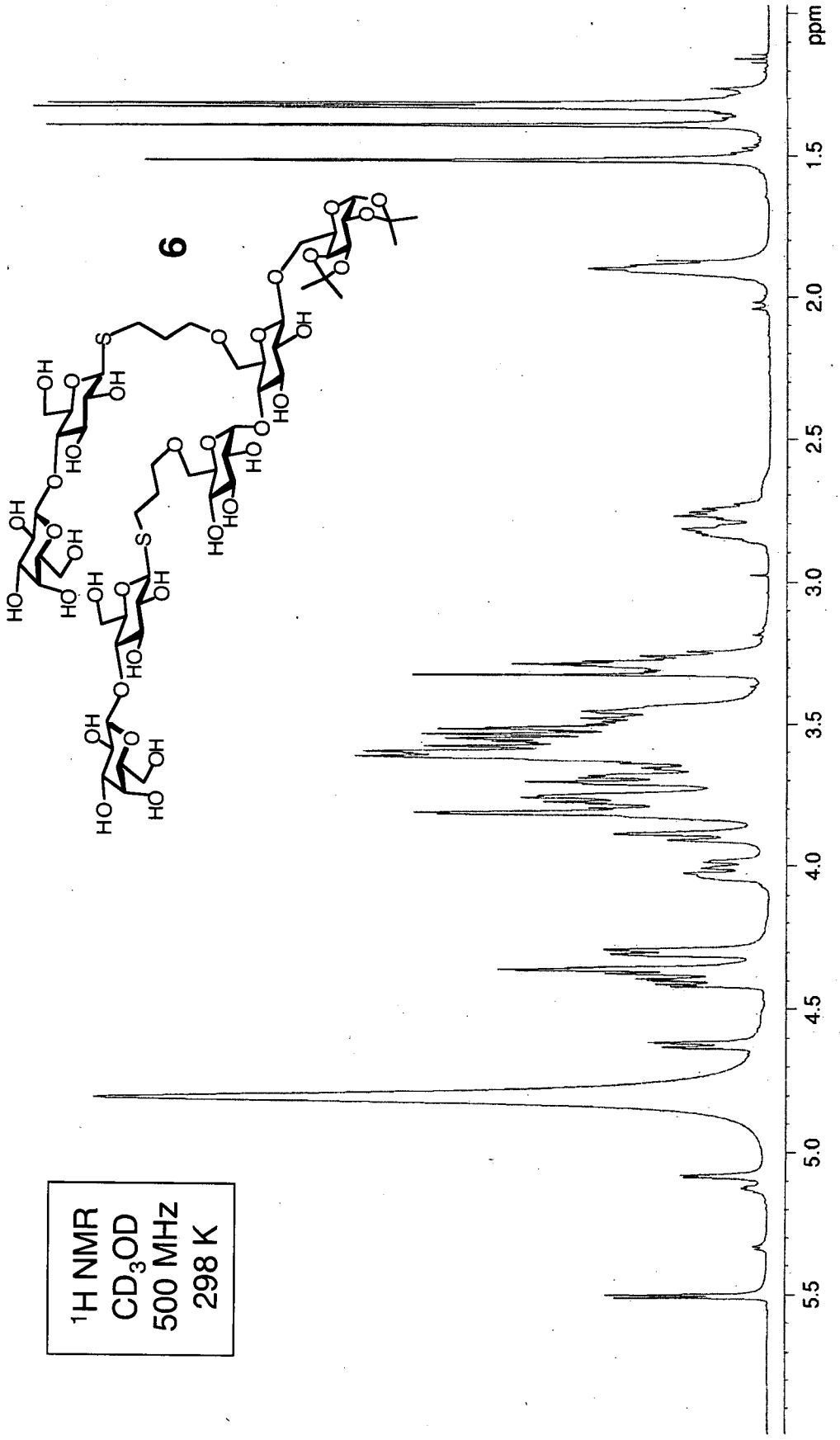
88.753


^{13}C NMR
D_2O
125 MHz
298 K
5
ppm


6
^{1}H NMR
CD_3OD
500 MHz
298 K
ppm
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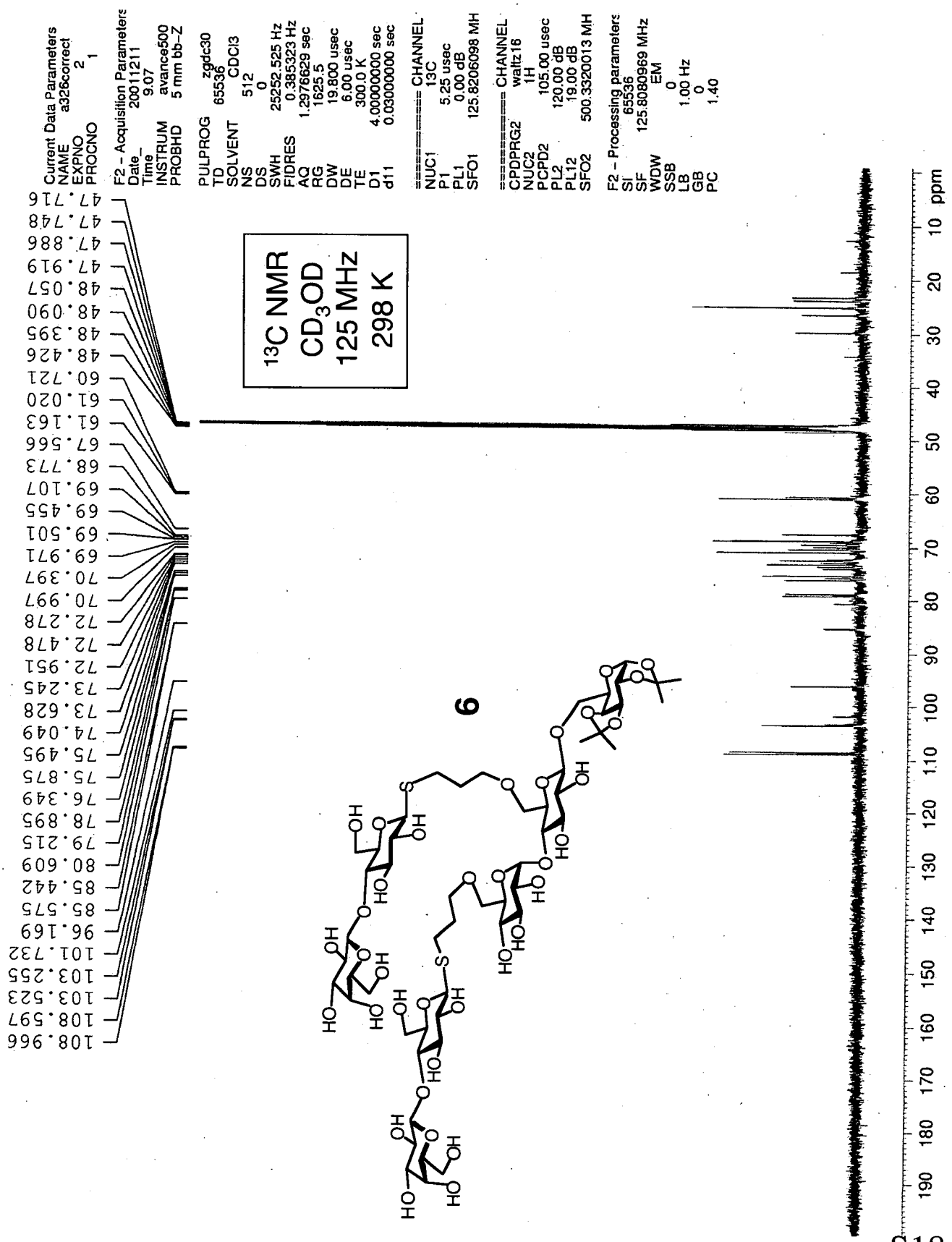
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5


13C NMR
CD3OD
125 MHz
298 K
6
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PULPROG zgdc30
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SWH 25252.525 Hz
FIDRES 0.385323 Hz
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RG 1625.5
DW 19.800 usec
DE 6.00 usec
TE 300.0 K
D1 4.00000000 sec
d11 0.03000000 sec
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P1 5.25 usec
PL1 0.00 dB
SFO1 125.8206098 MH
======== CHANNEL
CPDPRG2 waltz16
NUC2 1H
PCPD2 105.00 usec
PL2 120.00 dB
PL12 19.00 dB
SFO2 500.3320013 MH
F2 - Processing parameters
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SF 125.8080969 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
47.716
47.748
47.886
47.919
48.057
48.090
48.395
48.426
60.721
61.020
61.163
67.566
68.773
69.107
69.455
69.501
69.971
70.397
70.997
72.278
72.478
72.951
73.245
73.628
74.049
75.495
75.875
76.349
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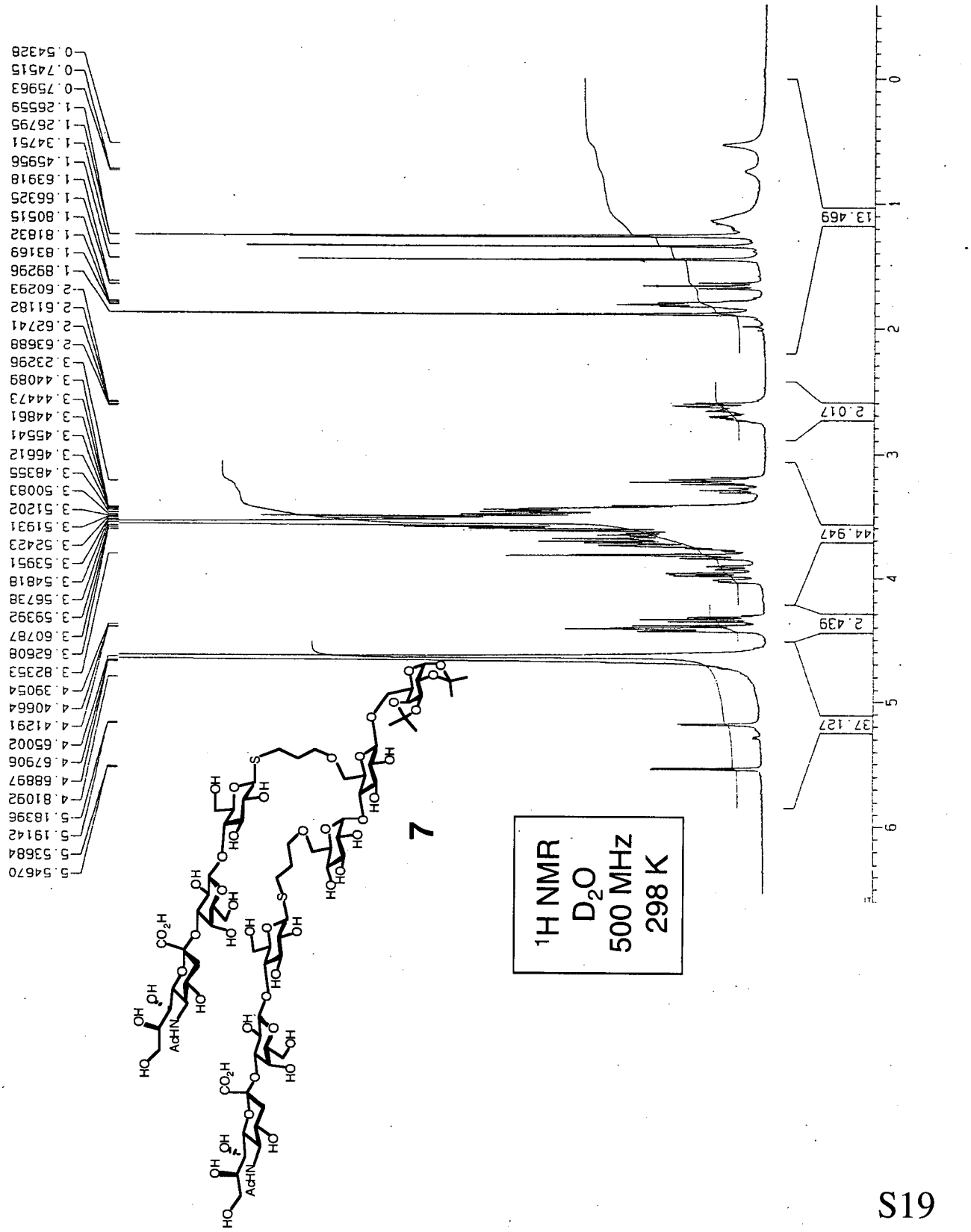

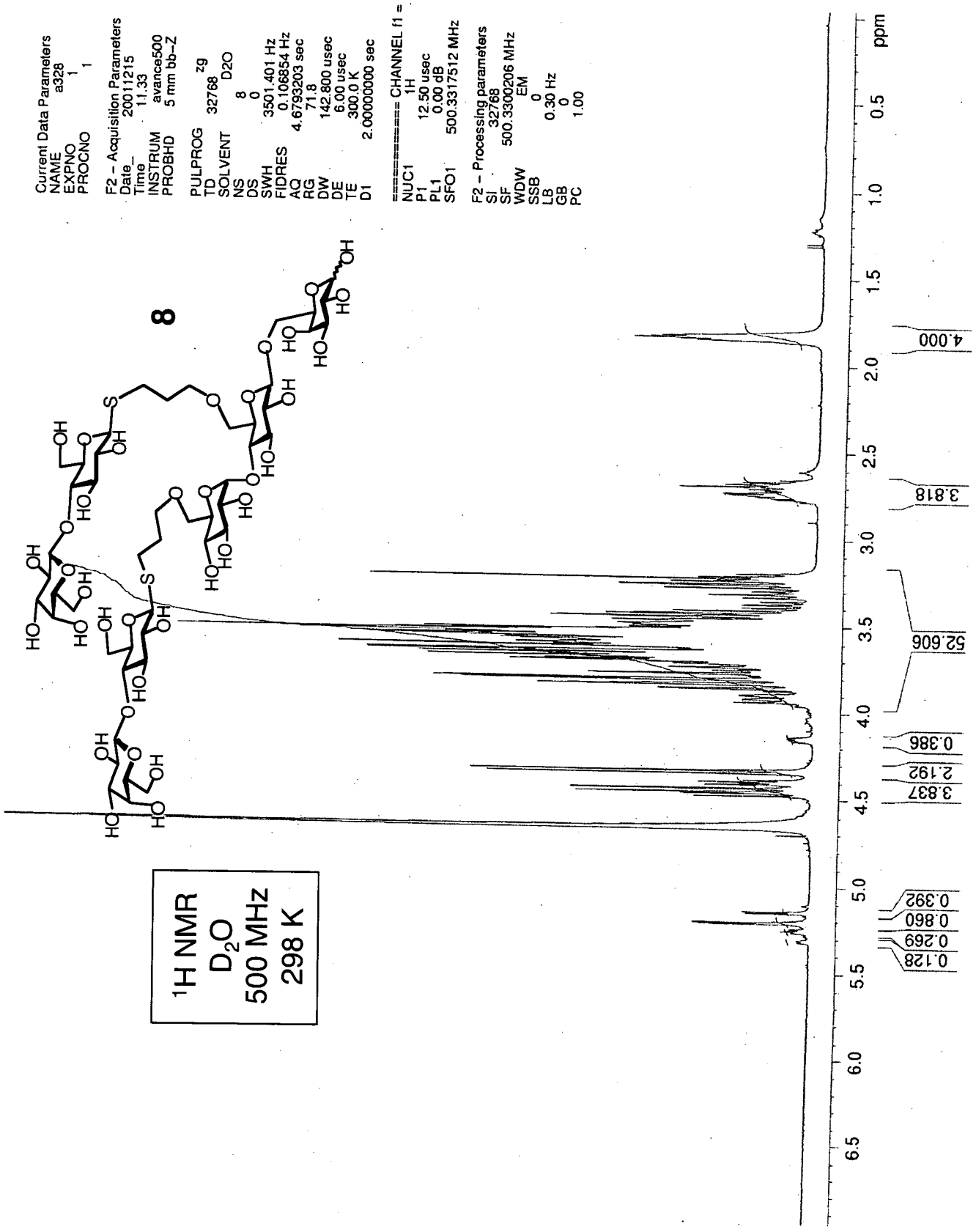
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PULPROG zg
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SWH 3501.401 Hz
FIDRES 0.106854 Hz
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DW 142.800 usec
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TE 300.0 K
D1 2.00000000 sec
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PL1 0.00 dB
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F2 – Processing parameters
SI 32768
SF 500.3300206 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8
^{1}H NMR
D_2O
500 MHz
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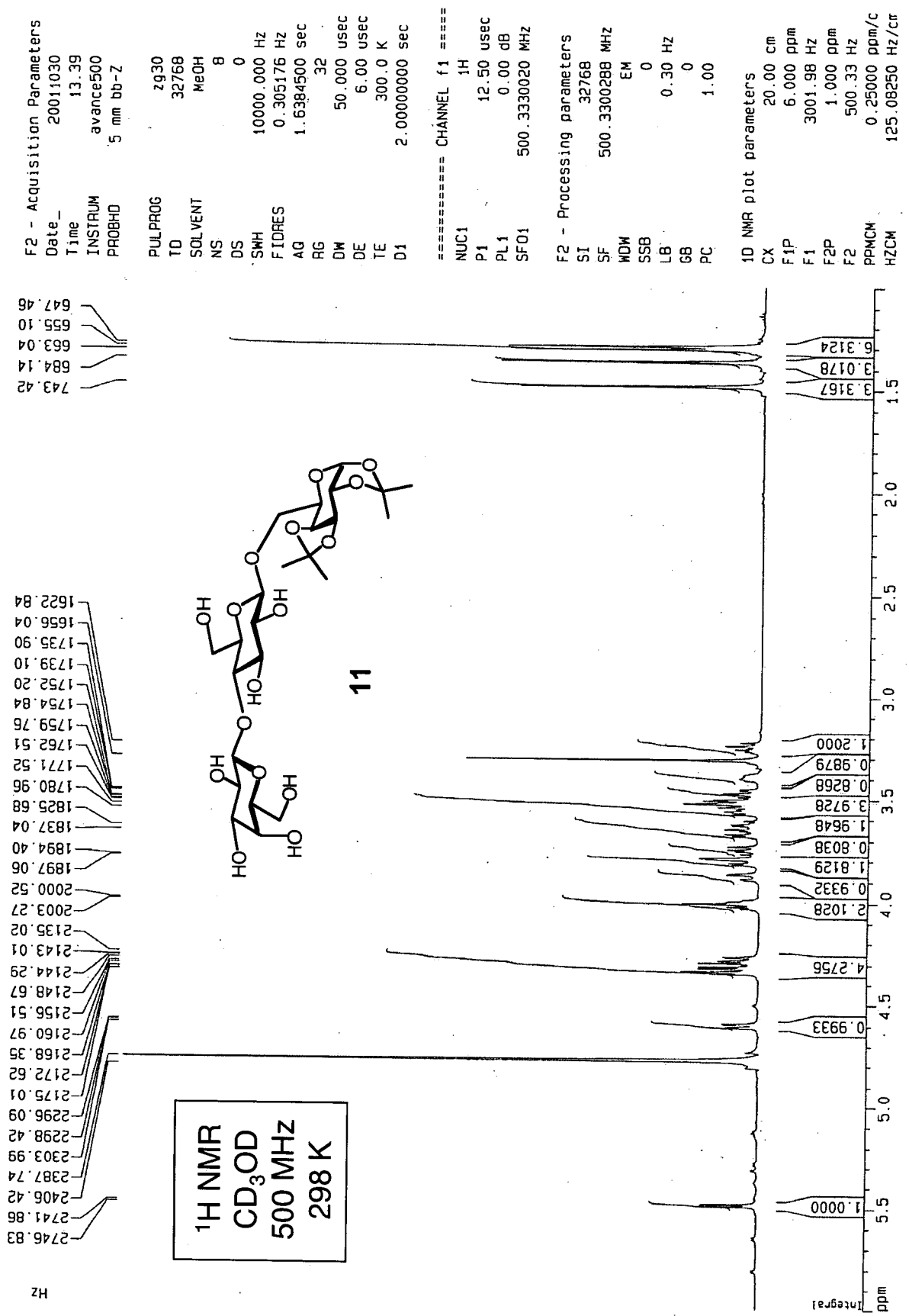
ppm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RG 32
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DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
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PL1 0.00 dB
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SI 32768
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WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1D NMR plot parameters
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F1 3001.98 Hz
F2P 1.000 ppm
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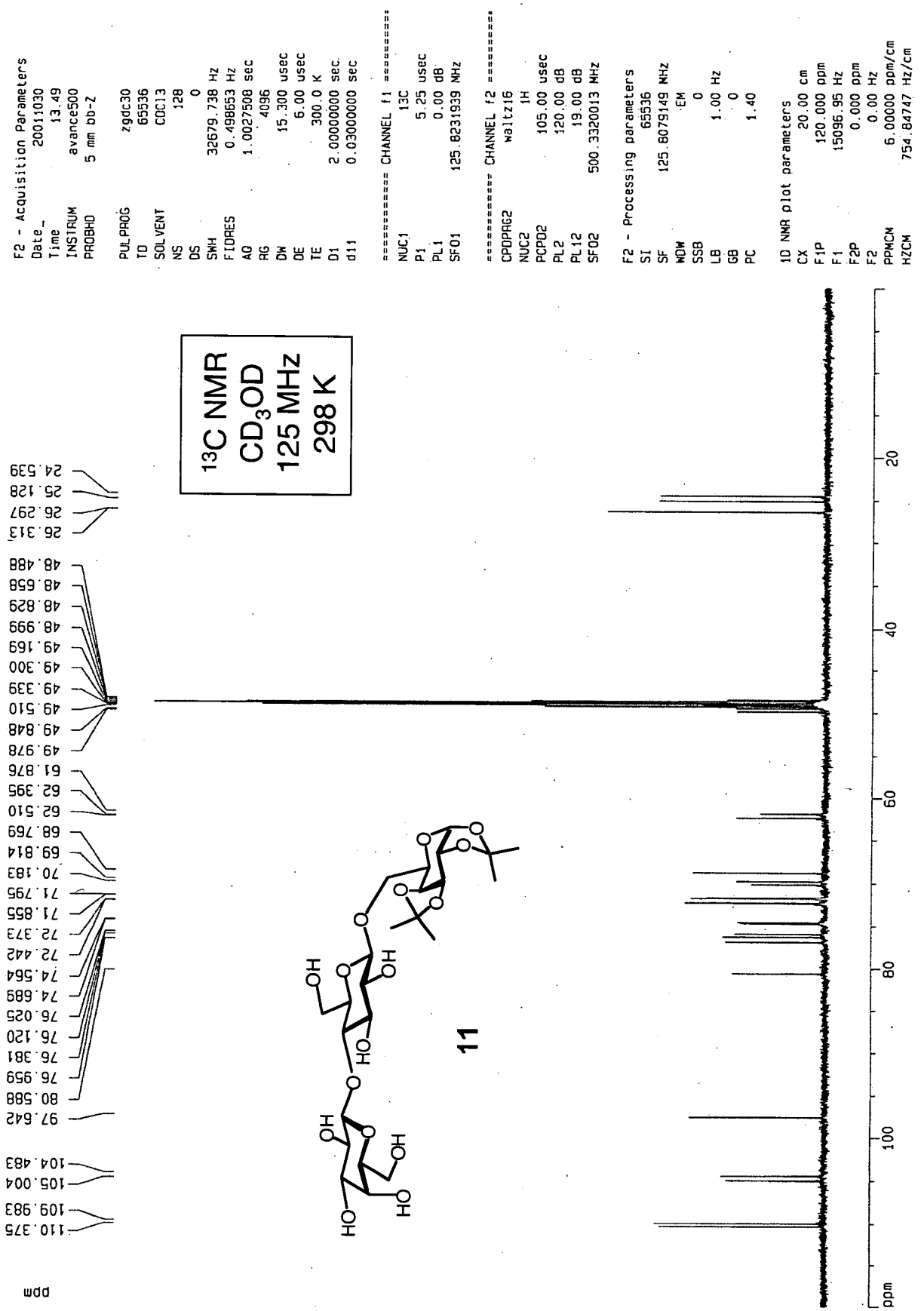
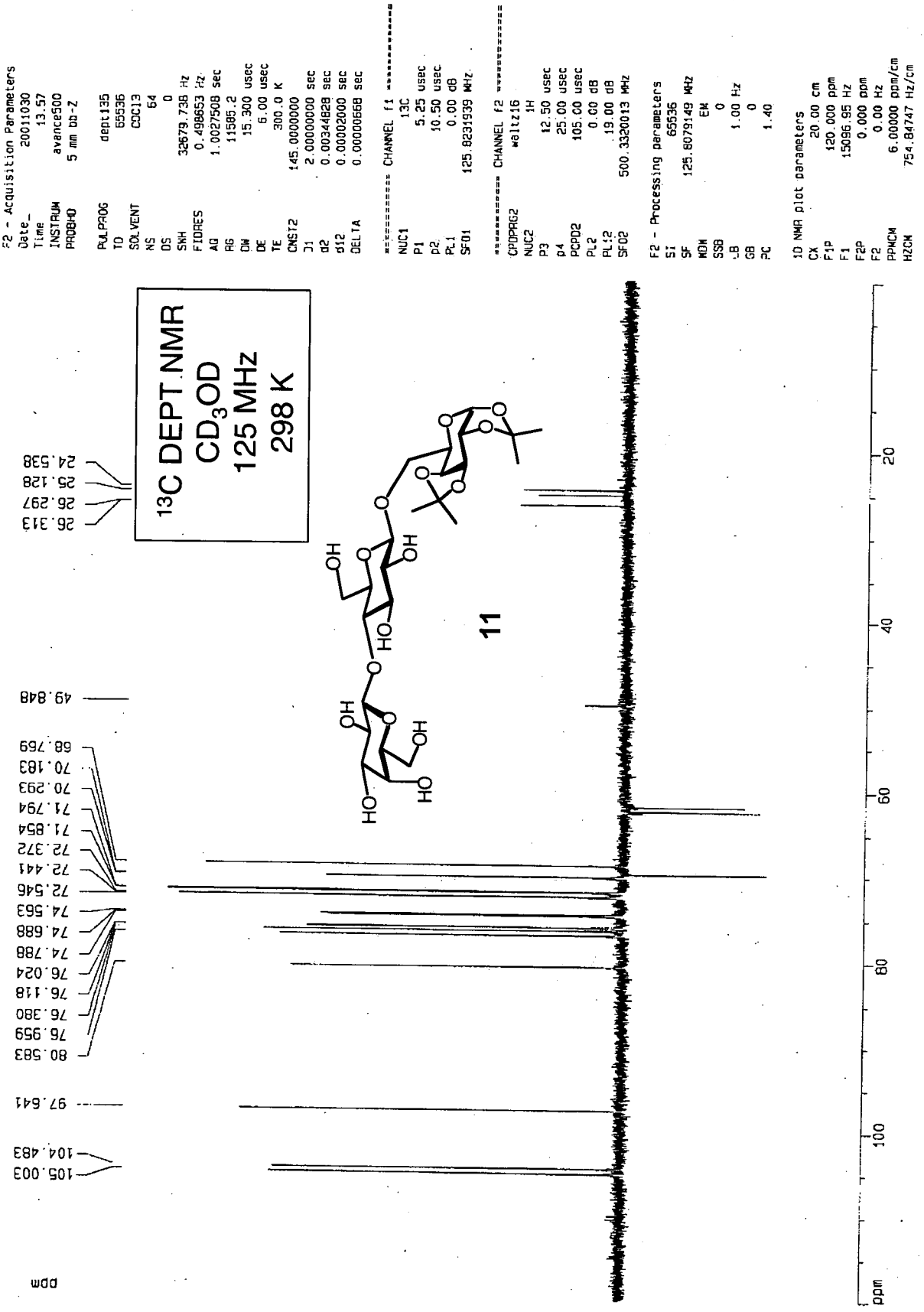
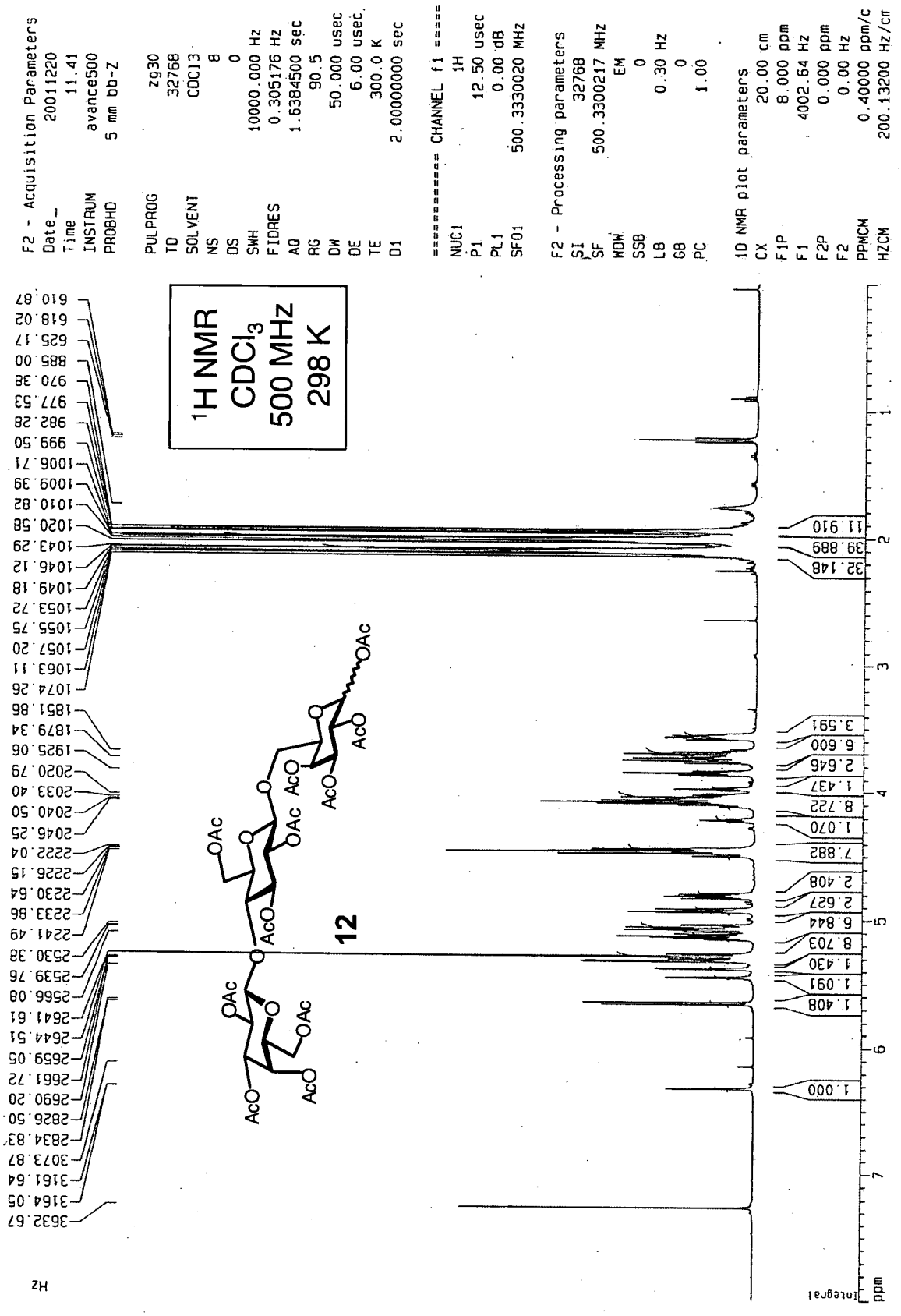
HZCM 125.08250 Hz/cm
1H NMR
CD3OD
500 MHz
298 K
11
Hz
ppm
Integral



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DS 0
SWH 10000.000 Hz
FIDRES 0.305176 Hz
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RG 90.5
DW 50.000 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
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PL1 0.00 dB
SFO1 500.3330020 MHz
F2 - Processing parameters
SI 32768
SF 500.3300217 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
F1P 8.000 ppm
F1 4002.64 Hz
F2P 0.000 ppm
F2 0.00 Hz
PPMCM 0.40000 ppm/c
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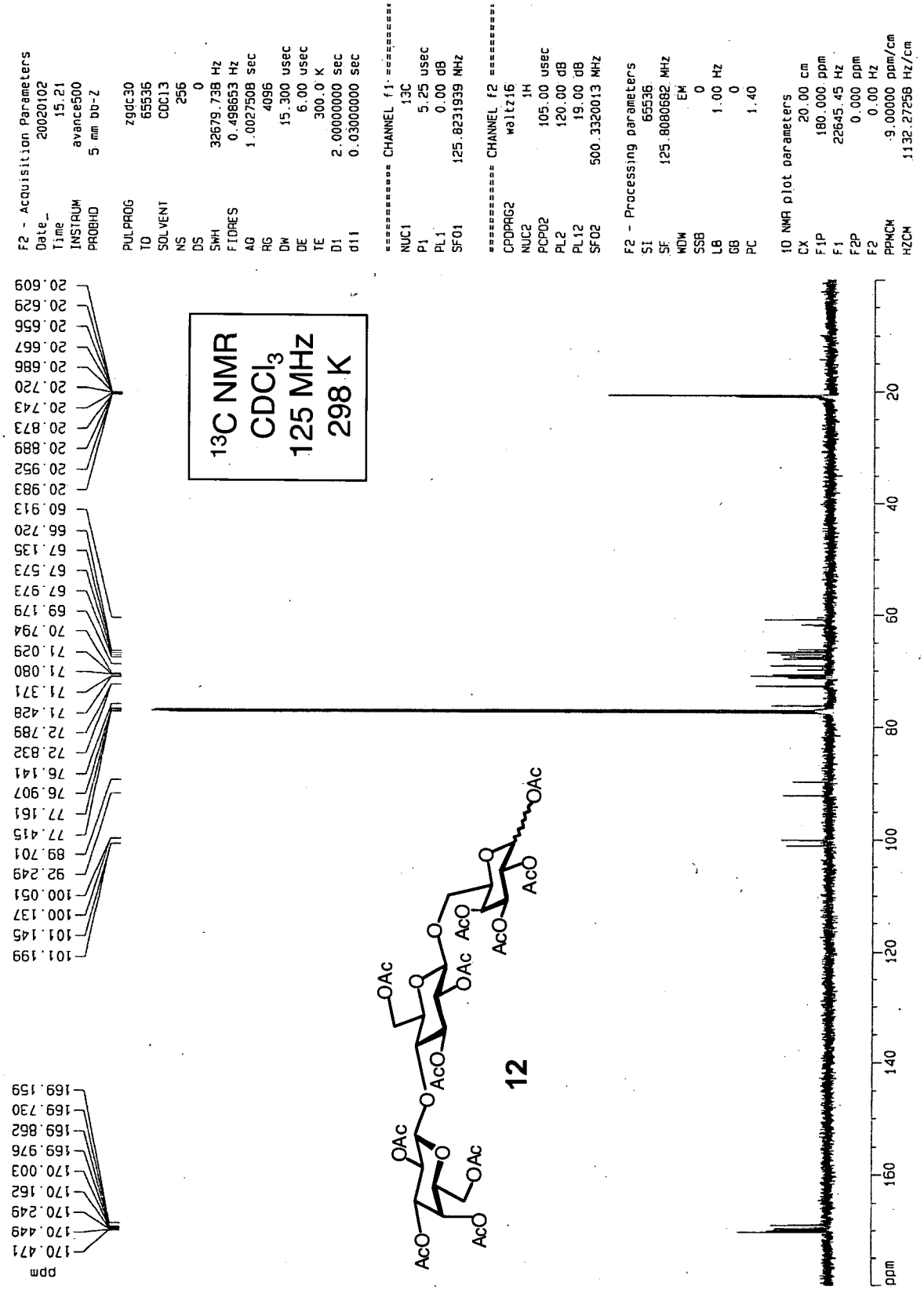
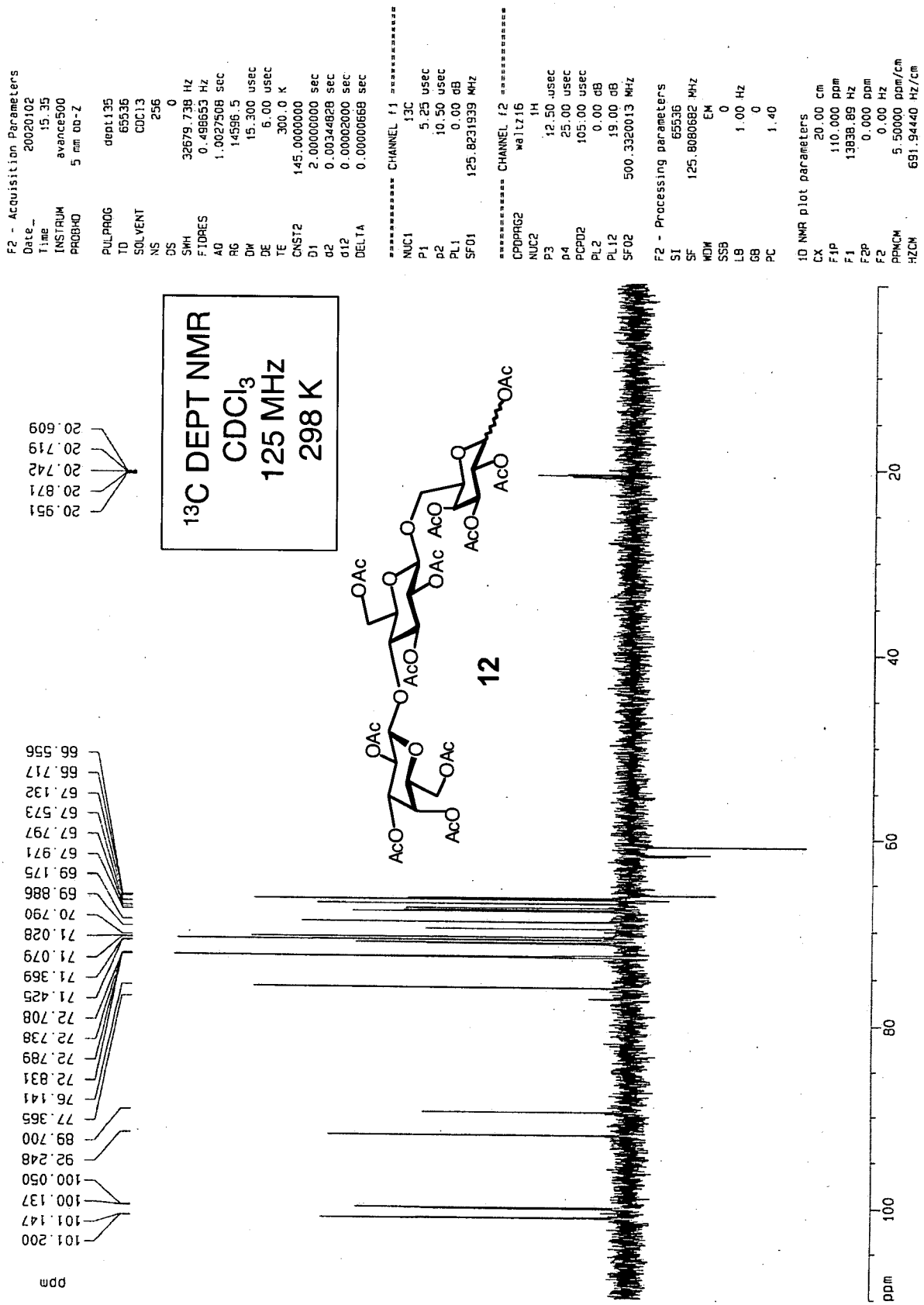
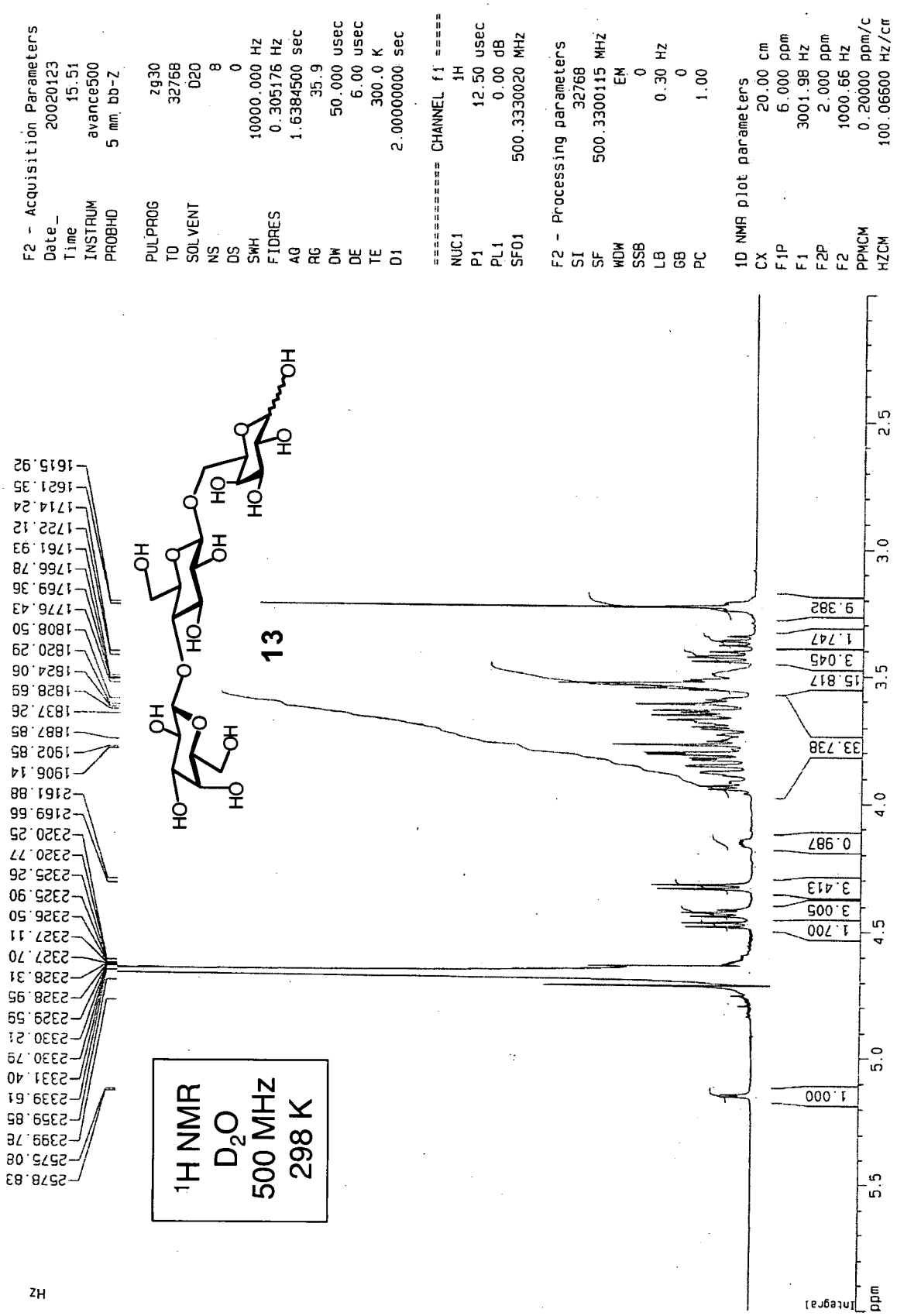
1H NMR
CDCl3
500 MHz
298 K
12
OAc
AcO
Hz
ppm
Integral



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SWH 10000.000 Hz
FIDRES 0.305176 Hz
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RG 35.9
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DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
========== CHANNEL f1 =====
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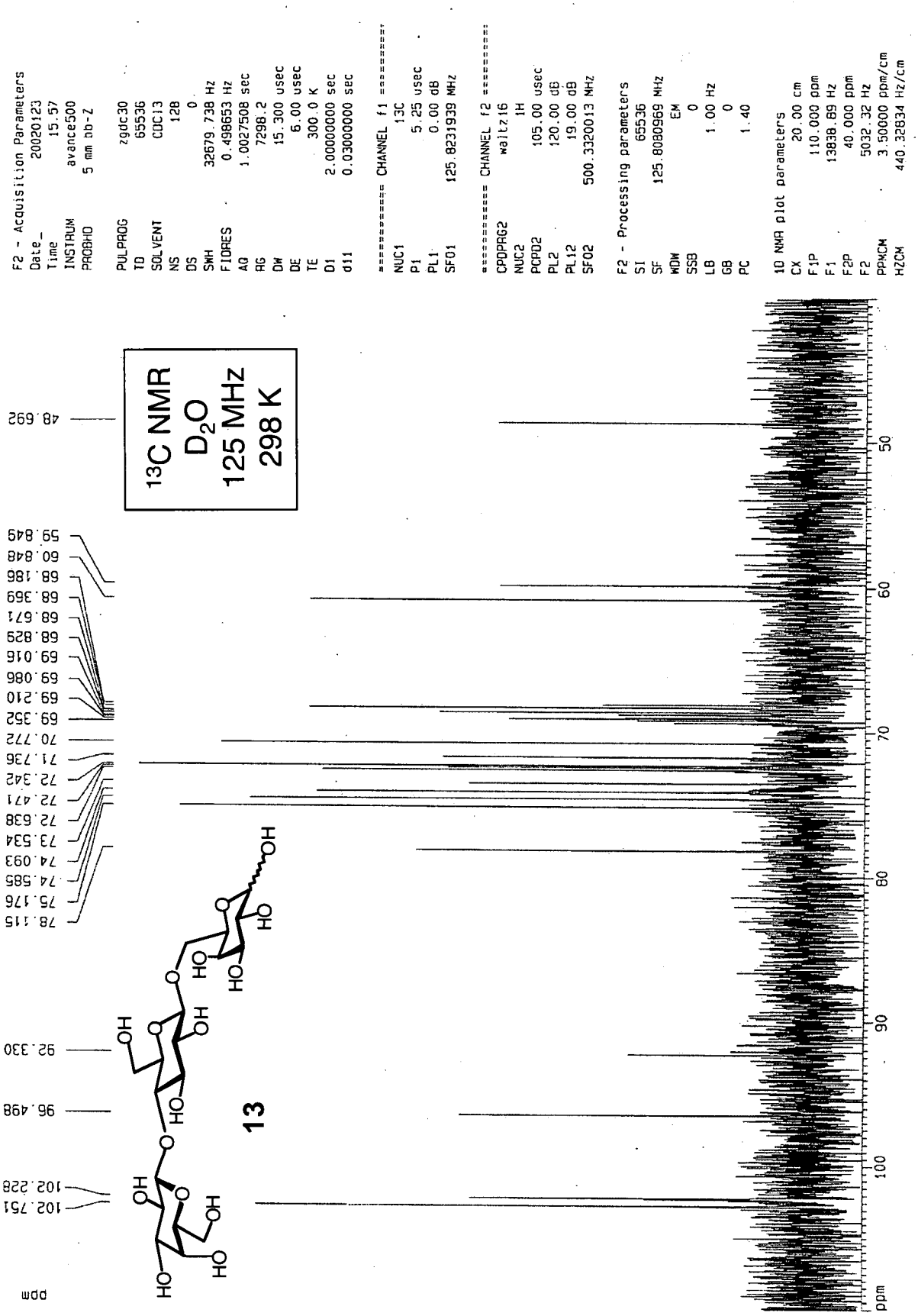
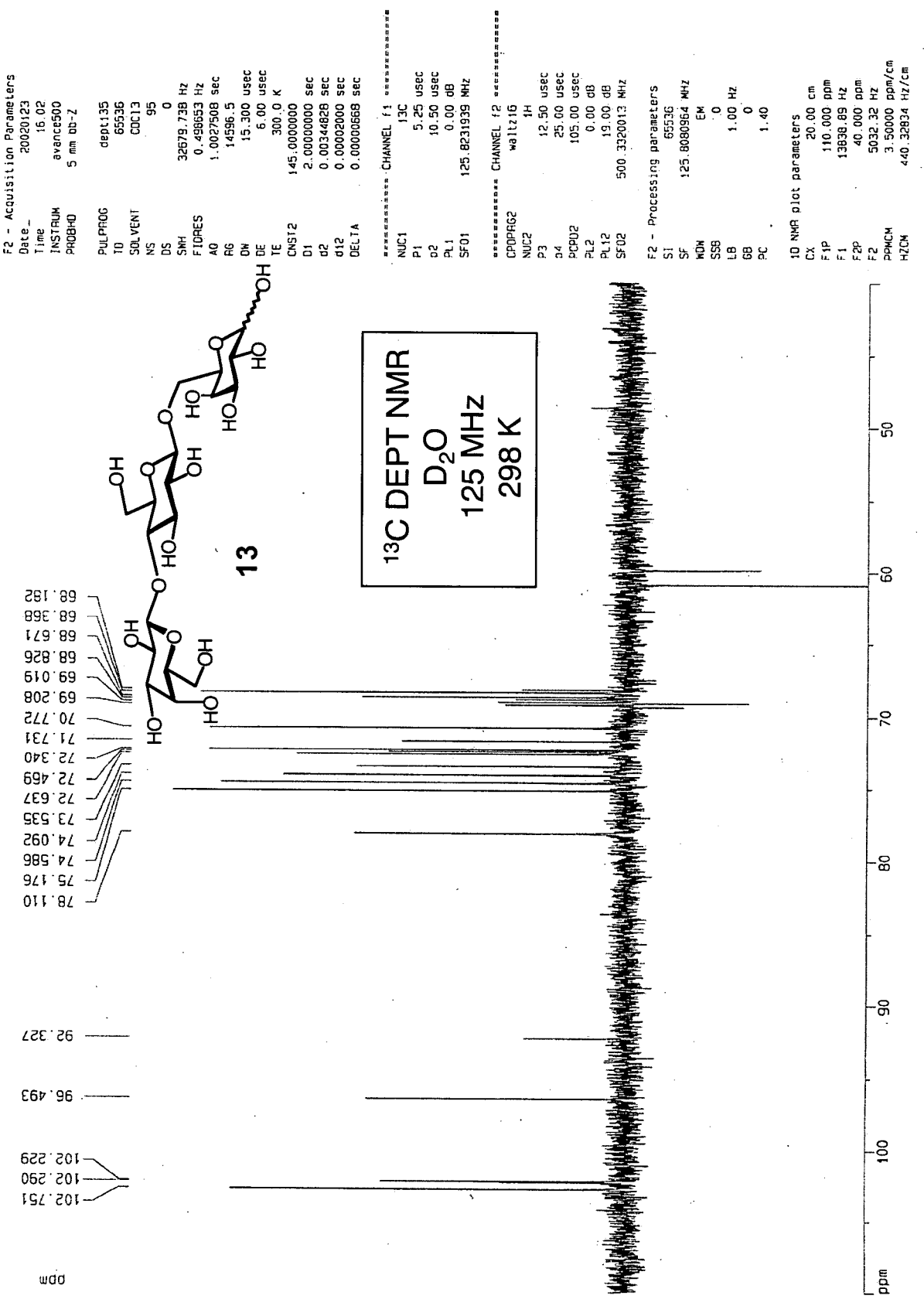
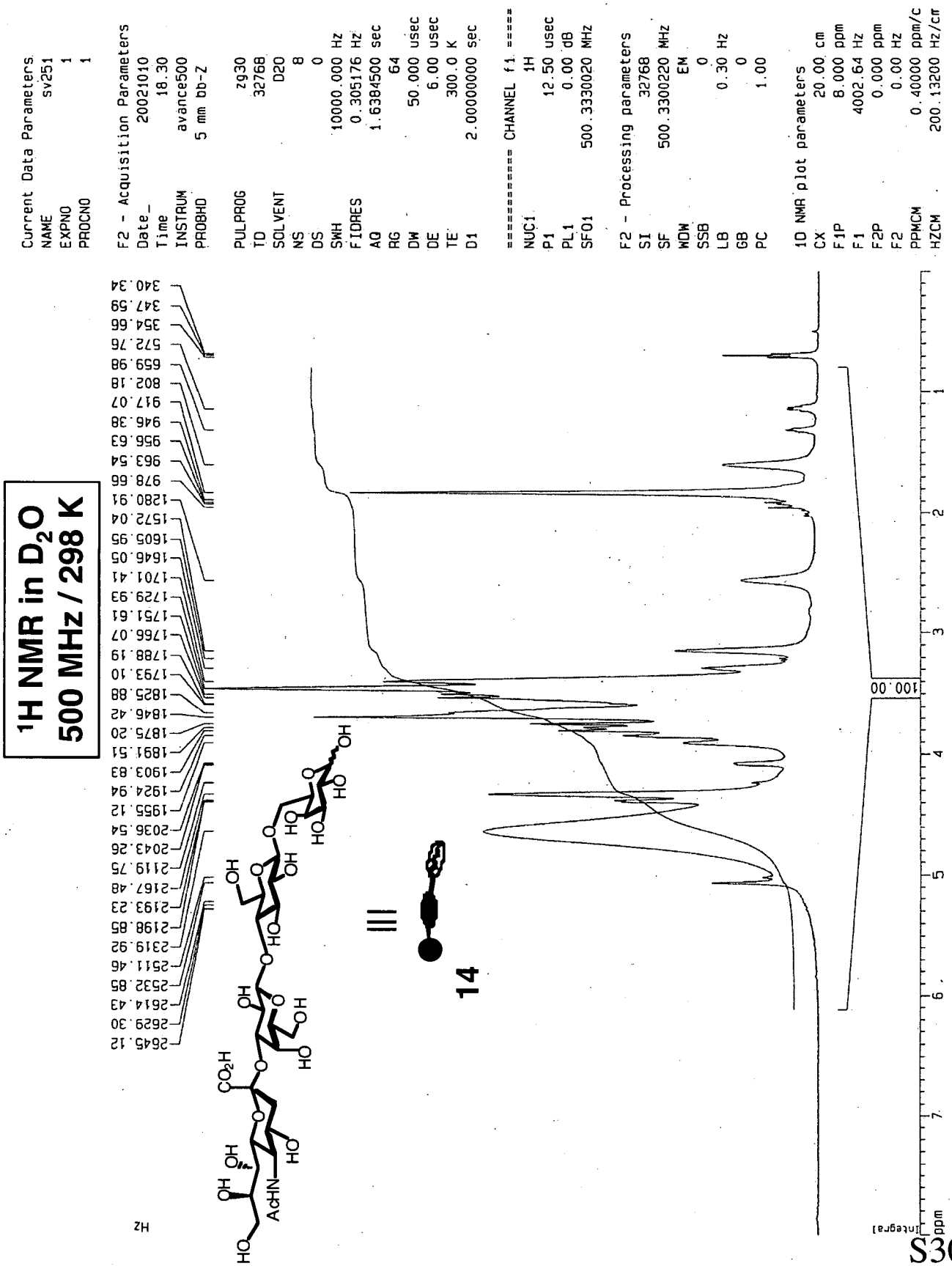
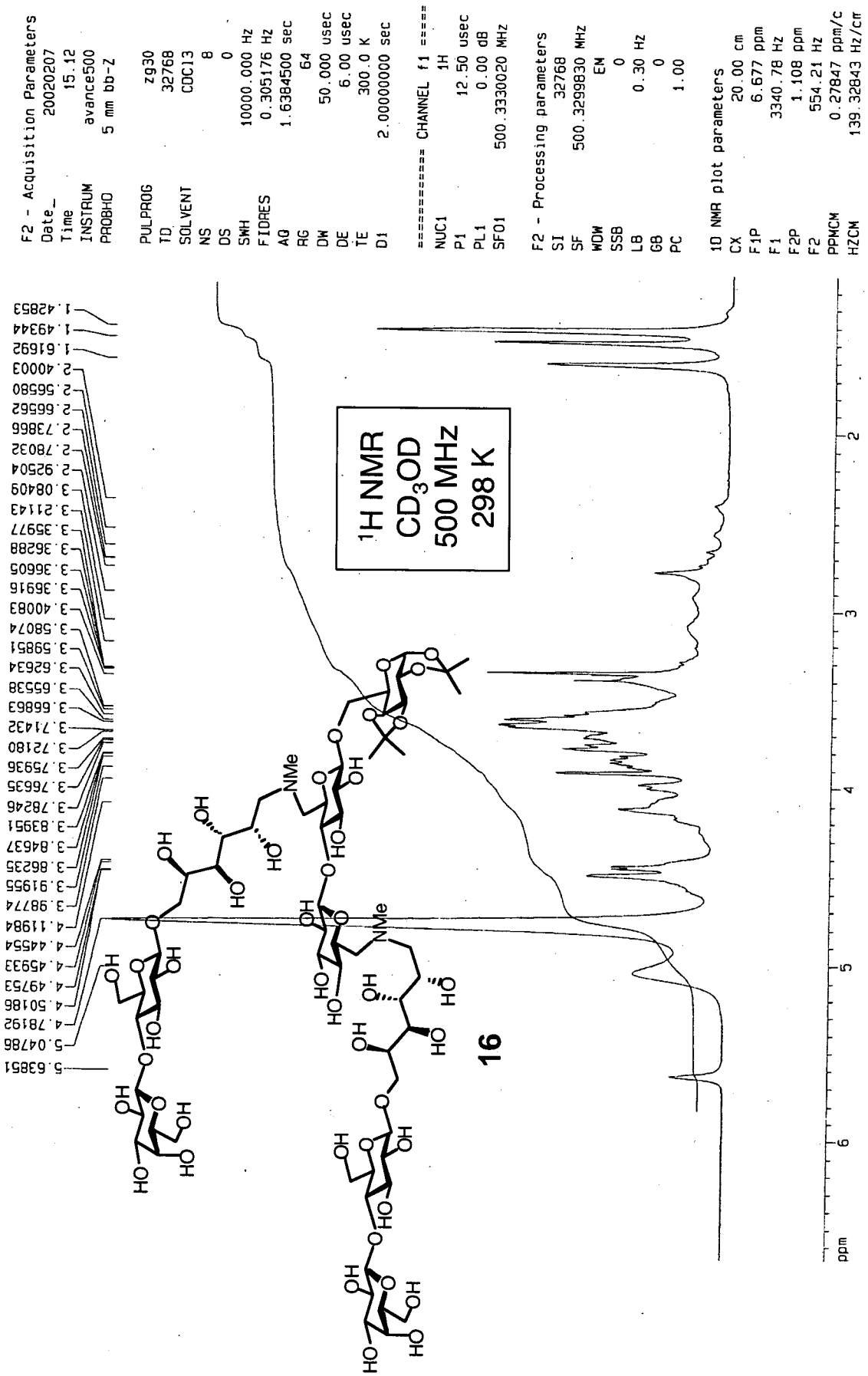
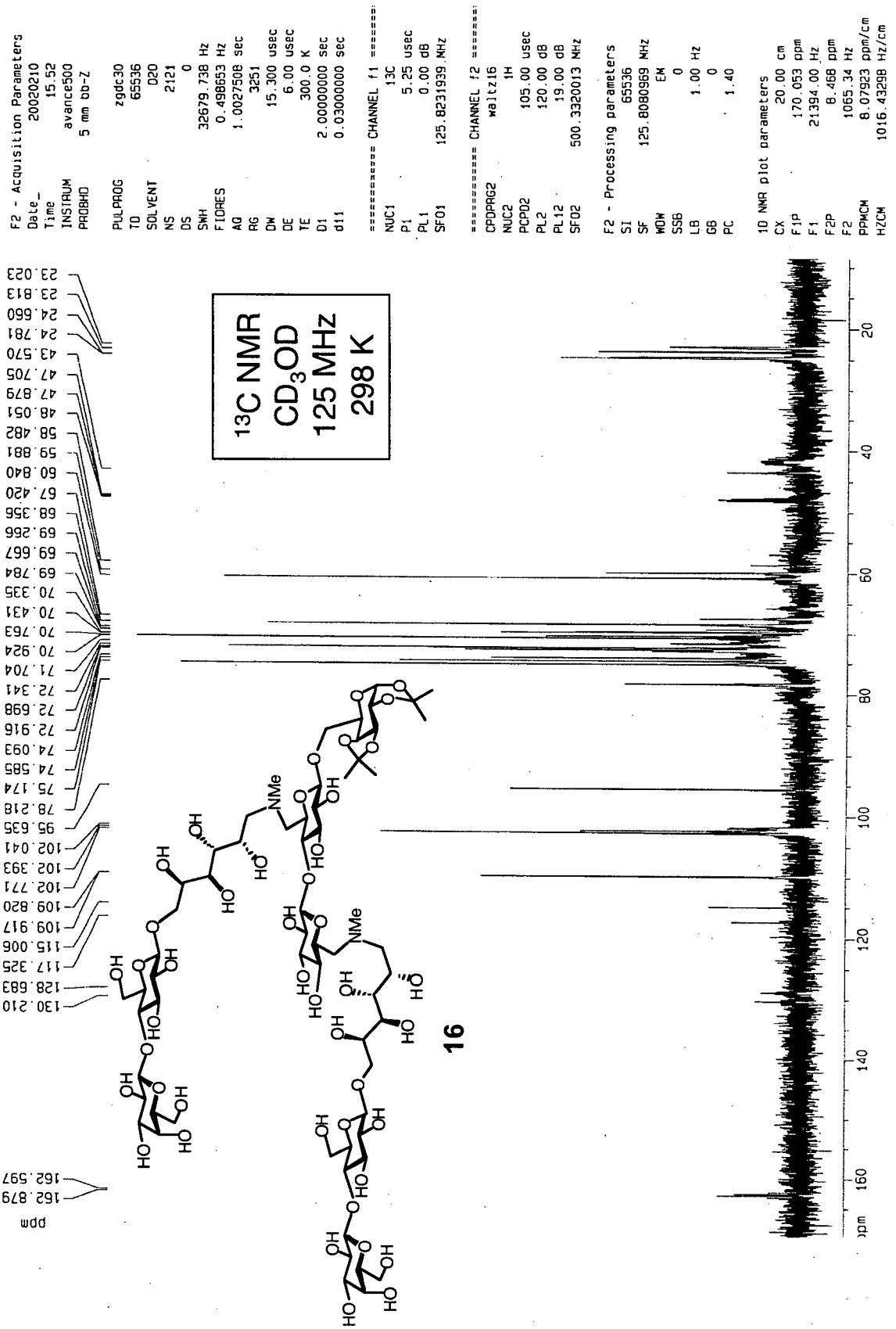
P1 12.50 usec
PL1 0.00 dB
SFO1 500.3330020 MHz
F2 - Processing parameters
SI 32768
SF 500.3300115 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
F1P 6.000 ppm
F1 3001.98 Hz
F2P 2.000 ppm
F2 1000.66 Hz
PPMCM 0.20000 ppm/c
HZCM 100.06600 Hz/cm
1H NMR
D2O
500 MHz
298 K
13
9.382
1.747
3.045
15.817
33.738
0.987
3.413
3.005
1.700
1.000
Integral
ppm
Hz

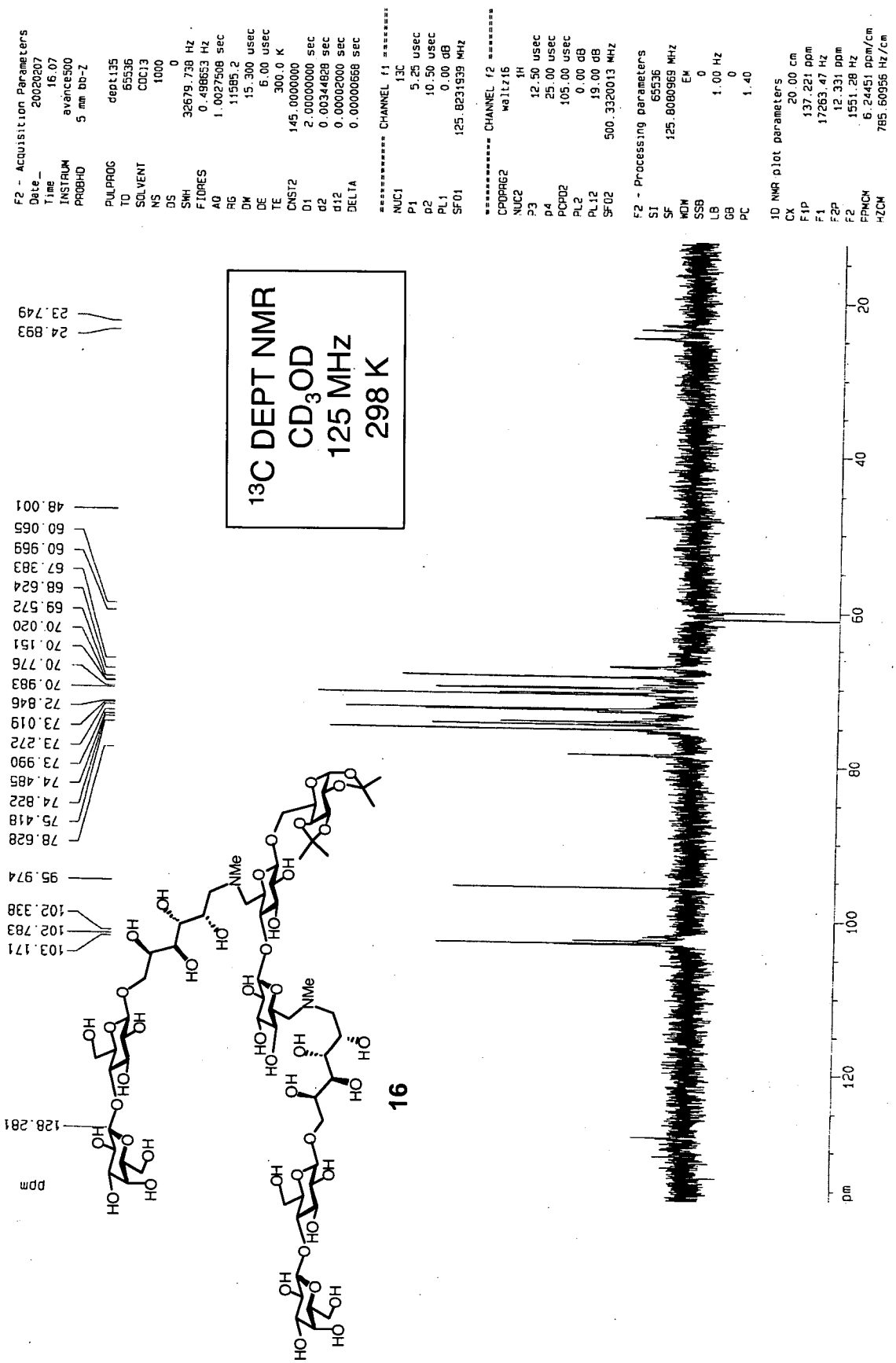

F2 - Acquisition Parameters
Date_ 20020123
Time 15.57
INSTRUM avance500
PROBHD 5 mm bb-Z
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 128
DS 0
SWH 32679.738 Hz
FIDRES 0.498653 Hz
AQ 1.0027508 sec
RG 7298.2
DW 15.300 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 5.25 usec
PL1 0.00 dB
SFO1 125.8231939 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 105.00 usec
PL2 120.00 dB
PL12 19.00 dB
SFO2 500.3320013 MHz
F2 - Processing parameters
SI 65536
SF 125.8080969 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 110.000 ppm
F1 13838.89 Hz
F2P 40.000 ppm
F2 5032.32 Hz
PPMCM 3.50000 ppm/cm
HZCM 440.32834 Hz/cm
13C NMR
D2O
125 MHz
298 K
48.692
59.849
60.848
68.186
68.369
68.671
68.829
69.016
69.086
69.210
69.352
70.772
71.736
72.342
72.471
72.638
73.534
74.093
74.585
75.176
78.115
92.330
96.498
102.228
102.751
13
ppm






F2 - Acquisition Parameters
Date_ 20020207
Time 15.12
INSTRUM avance500
PROBHD 5 mm bb-Z
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 8
DS 0
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 1.6384500 sec
RG 64
DW 50.000 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
========== CHANNEL f1 =====
NUC1 1H
P1 12.50 usec
PL1 0.00 dB
SFO1 500.3330020 MHz
F2 - Processing parameters
SI 32768
SF 500.3299830 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
F1P 6.677 ppm
F1 3340.78 Hz
F2P 1.108 ppm
F2 554.21 Hz
PPMCM 0.27847 ppm/c
HZCM 139.32843 Hz/cm
1.42853
1.49344
1.61692
2.40003
2.55580
2.66562
2.73866
2.78032
2.92504
3.08409
3.21143
3.35977
3.36288
3.36605
3.36916
3.40083
3.58074
3.59851
3.62634
3.65538
3.66863
3.71432
3.72180
3.75936
3.76635
3.78246
3.83951
3.84637
3.86235
3.91955
3.98774
4.11984
4.44554
4.45933
4.49753
4.50186
4.78192
5.04786
5.63851
1H NMR
CD3OD
500 MHz
298 K
NMe
NMe
16
ppm
1
2
3
4
5
6




^{13}C DEPT NMR
CD_3OD
125 MHz
298 K
16
ppm
pm
23.749
24.893
48.001
60.065
60.969
67.383
68.624
69.572
70.020
70.151
70.776
70.983
72.846
73.019
73.272
73.990
74.485
74.822
75.418
78.628
95.974
102.338
102.783
103.171
128.281
F2 - Acquisition Parameters
Date_ 20020207
Time 16.07
INSTRUM avance500
PROBHD 5 mm bb-Z
PULPROG dept135
TD 65536
SOLVENT CDCl3
NS 1000
DS 0
SWH 32679.738 Hz
FIDRES 0.498653 Hz
AQ 1.0027508 sec
RG 11585.2
DW 15.300 usec
DE 6.00 usec
TE 300.0 K
CNST2 145.0000000
D1 2.00000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
DELTA 0.00000668 sec
CHANNEL f1
NUC1 13C
P1 5.25 usec
p2 10.50 usec
PL1 0.00 dB
SFO1 125.8231939 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
P3 12.50 usec
p4 25.00 usec
PCPD2 105.00 usec
PL2 0.00 dB
PL12 19.00 dB
SFO2 500.3320013 MHz
F2 - Processing parameters
SI 65536
SF 125.8080969 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 137.221 ppm
F1 17263.47 Hz
F2P 12.331 ppm
F2 1551.28 Hz
PPMCM 6.24451 ppm/cm
HZCM 785.60956 Hz/cm